Herbivory and tannin polyphenols in mediterranean ecosystems

by

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Contents

1.0 Abstract

The dietary choice of some mammalian and insect herbivores was examined together with the nutritional chemistry of their foodplants in mediterranean-type ecosystems in South Africa, France and California. The general hypothesis underlying this study was that the very low soil nutrient levels in mediterranean ecosystems results in nutritional imbalances in the food plants available to herbivores both from shortages of beneficial components and from increases in deleterious secondary metabolites.

In South African shrubland, three species of commonly occurring endemic small antelope (Raphicerus campestris steenbok, R. melanotis grysbok and Sylvicapra grimmia duiker) fed selectively on or avoided approximately 25 shrubland species. Their species preferences, quantified by means of a Jacobs D index of selectivity, were not correlated with the relative abundance of these plants in the vegetation. Antelope shifted their preferences among different life-form categories coincident with phenological changes in the plant species in these categories. Observations of captive R. campestris and R. melanotis supported these findings. Twelve plant species were collected seasonally and assayed for different types of polyphenol compounds. The assays included analysis for total polyphenols, proanthocyanidins, flavanols and astringency. Selection of plant species included those that were abundant in the vegetation, or were obviously preferred or avoided. Mean seasonal values for all tests increased from lowest concentrations in winter to highest concentrations in autumn. Total polyphenols (TAE) for individual species ranged very widely from 2.0 to 32.0 % dry weight (d.w.). Concentrations in new and old leaves were not significantly different. Browse data, polyphenol data and data from proximate analyses (proteins, carbohydrates, fats, fibre and ash) were assembled in a matrix: stepwise regression

analysis of this data matrix indicated that total polyphenols and astringency were the strongest predictors of browse preference. This appears to be the first time that ruminant foraging preferences have been evaluated in this way. Vegetation biomass of this shrubland (1440 g m^{-2}) and production (360 g m^{-2} per year) were estimated from an exclusion plot experiment. These values are discussed in comparison with those from both mediterranean and other low nutrient ecosystems.

In southern France, garrigue plant species growing on a higher nutrient calcareous substrate had higher foliar N levels than the same species growing on a lower nutrient siliceous substrate(maquis). However the leaves of the maquis species had significantly higher levels of P, more water, higher tannin polyphenol concentrations and larger leaf areas. The amount of insect damage on garrigue and maquis plants was similar, presumably due to different nutritional "advantages" in each case. Artificial soil fertilization significantly elevated N levels in Q. coccifera and increased total leaf areas, and these leaves had significantly more insect damage. A combination of controlled stock utilization and artificial fertilization significantly lowered the levels of condensed tannins in Q. coccifera and raised the levels of foliar N. Some effects of burning on Q.coccifera are also described. This evidence from garrigue and maquis in southern France suggests that the chemical profile of plants in these shrublands is fairly plastic.

In two evergreen (Q. agrifolia and Q. durata) and three deciduous Californian oak species (Q. lobata , Q. douglasii and Q. kelloggii) total polyphenol concentrations varied from 8.5 % d.w. (TAE) in Q. kelloggii in late summer to 27.5 % d.w. (TAE) in Q. durata in late summer during an outbreak of a lepidopteran herbivore, Phryganidia californica . Condensed tannins increased in all species from spring to late summer. In a comparison of larval mass of P. californica collected from all five oaks simultaneously,

larvae growing on evergreen oaks were significantly smaller than those growing on deciduous oaks. Stepwise regression analysis indicated that phosphorus and astringency most strongly predicted caterpillar mass. Tannin polyphenols and nutritional components of the leaves of one evergreen and one deciduous species were measured at regular intervals for 15 months during the Phryganidia outbreak. Two complete defoliations of Q agrifolia induced these trees to produce three cohorts of new leaves during that season. The deciduous Q. lobata were defoliated once by the end of the growing season and some trees produced new leaves before leaf abscission. In Q. agrifolia highest mean levels of tannin polyphenols were 21.0 % d.w. (TAE) and declined to a minimum of 11.0 % d.w., and in Q. lobata maximum mean levels were 18.5% d.w. (TAE) and declined to 12.0% d.w. before abscission: this is contrary to findings from other oak - lepidopteran studies. Condensed tannins generally increased in both species as leaves aged. There was no evidence for an inductive effect in subsequent cohorts of leaves. Leaf nitrogen levels declined from a maximum in new leaves of 3.0 to 4.0 % d.w. in both species to approximately 1.0 % d.w. in Q. agrifolia and 2.0 % d.w. in Q. lobata .

These findings contribute significantly to knowledge of the ecological biochemistry of plant-animal interactions in three major areas: food quality and ruminant food preferences, changes in food quality during insect population outbreaks and the sensitivity of food quality to differences in substrate nutrient levels.

2.0 Acknowledgements

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3.0 Introduction

One major difference between plants and animals is in the relative importance of carbon and nitrogen in primary structural tissues. Animals both contain more nitrogen(N) (8 -14% dry weight (d.w.)) and require a high N intake to balance excretion losses. Plants, however, contain much lower levels of N (0.03 - 7.00 % d.w.) and depend instead on carbon in carbohydrates to build structural tissue (Mattson 1980). This difference is particularly important for herbivorous animals. They must compensate for these low nitrogen levels in their dietary plants, while coping with an obvious upper limit to their rates of food intake. This relationship has been examined both in invertebrates (Dadd 1973, White 1974, White 1984, McNeill & Southwood 1978) and vertebrate herbivores (Sinclair 1975,Westoby 1974, Smith et al. 1975, White 1978, Holter et al. 1979).

In plants the complex process of carbon gain from the atmosphere is closely linked to nutrients absorbed from the soil (Mooney 1972, Chapin 1980a). In particular nitrogen plays a key role in carbon gain capacity of leaves (Mooney 1972, Mooney & Gulmon 1982) hence the importance of carbon : nitrogen ratios in nutrient cycling and nutrient-use efficiency studies (Vitousek 1982). Photosynthesis converts this atmospheric carbon dioxide into sugar phosphates. These phosphates are then transformed into a diverse array of carbohydrates and derivatives. One of these, phosphoenol - pyruvate (PEP), can feed directly to the shikimate pathway and to the biosynthesis, in turn, of a wide variety of products (Mooney 1972). Many of these are classified as secondary compounds or allelochemicals (Harborne 1972, 1982, Swain

1979). During the last two decades an enormous amount of research has been published on the role of these compounds in plant - animal interactions (eg. Rosenthal & Janzen 1979 and references therein). Examples of secondary metabolites derived from shikimate include simple phenolics, polyphenolics (tannins) and lignins (Swain 1979), aromatic amino acids (Beevers 1976), coumarins (Murray et al. 1982) and flavonoids (Harborne 1979). As the term 'secondary ' implies , these compounds generally have little to do with primary metabolic processes. Until approximately 20 years ago, when their ecological importance became apparent (Fraenkel 1959), those that had been identified were considered to be merely metabolic waste products (Muller 1969). They generally contain very high proportions of carbon in contrast with animal waste products, as mentioned above.

A major functional effect of many secondary compounds is the deterrence of animal herbivores and micro-organisms on both terrestrial (Swain 1979) and marine plants (Lubchenco & Gaines 1981). Freeland and Janzen (1974) estimated that there were more than 12 000 low molecular weight compounds that can interfere with growth, neurological and tissue function, reproduction and digestion. The prevalent groups are soluble phenolics, alkaloids and terpenoids. Based on mode of physiological action, plant secondary metabolites have been divided broadly into two groups (Rhoades & Cates 1976, Feeny 1976): digestibility reducing compounds which are generally not lethal to a wide range of herbivores but which may be debilitating with protracted ingestion (Morton 1979) and toxins which are lethal to all but a specialised few which are able to detoxify them. Digestibility reducing compounds are thought to form indigestible complexes with proteins and are then excreted by the animal with a net loss of nitrogen (Robbins 1983). These complexes may form during mastication when plant cell walls and storage vacuoles are ruptured and proteins are then combined with these

products, or later in the gut, where they may interfere with microbial and/or enzyme action. In contrast, toxins rapidly pass through the gut wall, enter the blood stream and, unless detoxified, can cause organ failure or interfere with neurotransmission. A number of recent major reviews have synthesized information on the interplay between animal metabolism and plant chemicals in this rapidly expanding field (Rosenthal & Janzen 1979, Harborne 1982, Denno & McClure 1983). Most theory and research has been developed around insect herbivores (eg. Beck & Reese 1976, Feeny 1976, Rhoades & Cates 1976) but substantial and advanced work has also been done on mammalian herbivores (eg. Bryant & Kuropat 1980, McKey et al. 1981).

One particularly interesting group of chemicals is tannin polyphenols. These are generally classified as digestibility-reducing compounds although there is evidence that smaller hydrolysable tannins act more like toxins in vertebrates by being absorbed and then cause extensive physiological damage (Mould & Robbins 1982). Tannins affect a wide range of organisms including insects and mammals (Swain 1979). They have been detected in vast numbers of plant species (Bate-Smith 1954, 1962, Bate-Smith & Metcalfe 1957, Swain 1979). Rhoades and Cates (1976) estimated that tannins occur in 17% of non-woody annuals, 14% of herbaceous perennials, 79% of deciduous woody perennials and 87% of evergreen woody perennials.

Soil nutrient levels have important effects on different facets of leaf chemistry and may thereby affect plant defenses against herbivores. Firstly, foliar N levels seem to reflect soil nutrient levels. The five mediterranean-type zones have some of the lowest soil nutrition levels in the world (Specht & Moll 1983) and those in southern Africa are amongst the lowest of these (Di Castri 1981). Mooney and Gulmon (1982) compared nitrogen content and the degree of sclerophylly in leaves from sixteen tropical and

temperate communities. They found that South African fynbos leaves had the lowest N (6 mg g^{-1}) and highest leaf specific weights (LSW) (320 g m^{-2}) of all. At the other extreme, the leaves of some Californian annuals had five times more N (25 mg g^{-1}) and one-eighth the LSW (40 g m^{-2}) of fynbos leaves. Second, in some other studies low substrate nitrogen levels stimulated an increase in the biosynthesis of some phenolics (chlorogenic acid, coumarins and tannins) in intact plants (Davies et al. 1964, Del Moral 1972) and in tissue culture (Westcott & Henshaw 1976). Drought has also been associated with increased levels of phenolics in sunflowers (Del Moral 1972). Summer drought is a particular feature of mediterranean climate zones.

In general, severe defoliation is detrimental to plant fitness and this has been documented for wild trees and shrubs, agricultural crops and in biological control of weeds (Krischik & Denno 1983 and references therein). Plant fitness is affected despite the well known abilities of many plants to compensate for loss of photosynthetic tissue. Because of this selection may magnify the imbalance between defensive and nutritional chemicals even further, and this would serve to protect these plants. Therefore, herbivores in mediterranean ecosystems may have to cope with especially high levels of carbon-containing secondary compounds in order to obtain sufficient nitrogen from plant material.

Herbivory and the interaction between plant nutrition qualities and defensive secondary metabolites is the major theme of this thesis.

4.0 Mediterranean ecosystems

Mediterranean ecosystems with their distinctive climates occur mostly on the western coastal margins of the six continents at 30° to 40° latitudes in both hemispheres (Aschmann 1973). The general descriptive name is derived from those surrounding the Mediterranean sea; the other ecosystems are found in California, Chile, South Africa and Australia. They are probably the most spatially restricted climate zones or ecosystem types on the planet, covering between 0.5 and 1.2 % of land area (Di Castri 1981). Their characteristic climate of hot, dry summers and mild, wet winters depends mostly on the existence of adjacent cold ocean currents flowing towards the equator parallel to the continental margins. In a geological time frame, mediterranean climates are very young, originating from the Pliocene 3 million years ago (Axelrod 1973, Deacon 1983). There are other similarities: Australia and South Africa have sheared continental margins and are ancient, stable basement systems, while Chile, California and the mediterranean basin have tectonically unstable mountain chains with volcanic activity (Deacon 1983). In contrast with climate and geology, there is much more heterogeneity in soil types, plant and animal communities (Cody & Mooney 1978, Milewski 1983). Soils vary from being moderately fertile (Chile, California) to having very low nutrient levels (Australia and South Africa), particularly with regard to nitrogen and phosphorous (Figure 4.0.1, Table 4.0.1).

Schimper (1903) was the first to bring attention to the similarities of these five mediterranean-type ecosystems. Plant biogeography and evolution has been widely reviewed for mediterranean ecosystems in both the northern hemisphere (Axelrod 1973, 1975, Raven 1973) and southern hemisphere (Deacon 1983). Succession, and the role of fire, have probably received the most attention of all areas of research in mediterranean ecosystems (Braun - Blanquet 1936, Mooney & Conrad 1977, Trabaud

& Lepart 1980 and references therein, Trabaud 1981). This is to be expected in view of the protracted impact of human occupation in some mediterranean zones.

Existing plant species distributions and community structure in all mediterranean climate zones should be strongly influenced by soil water relations (precipitation balanced against evapo-transpiration) (Miller 1983). For example, in evergreen dominants, carbon gain in one year depends heavily on the rainfall of the previous winter (Mooney 1972). Since carbon gain and allocation patterns are also intimately linked to nutrient availability (Mooney & Gulmon 1982), there appears to be a clear association between plant-species distribution, structure and phenophase activity on the one hand with soil nutrient chracteristics on the other, particularly in South Africa, Australia and Israel (Specht & Moll 1983, Rabinovitch-Vin 1983). On this basis Specht & Moll (1983) have suggested that it is possible broadly to distinguish shrublands on moderately leached soils from heathlands on strongly leached soils.

Much less research has been published on animal communities in mediterranean ecosystems. Community structure, diversity and biogeography of birds appears to have received most attention (Cody 1973, Cody & Mooney 1978, Blondel 1981, Cody 1983 but see Blondel 1984, Schodde 1981, Siegfried & Crowe 1983, Milewski 1983). Other published vertebrate studies include work on lizards in Chile (Fuentes 1981, Hurtubia & Di Castri 1973, Sage 1973), mammals (Kikkawa et al. 1979, Newsome & Catling 1979) and small mammals in Australia (Fox 1983, Braithwaite et al. 1978), and a review of South African fynbos vertebrates (Bigalke 1979). Published invertebrate studies include research on soil beetles in California, Chile, Europe and South Africa (Sáiz 1973), pseudoscorpions in all five mediterranean regions (Vitali-di Castri 1973) and soil fauna in Chile (Di Castri & Vitali-di Castri 1981).

Very little research has been published on the interaction of the plants and animals in Mediterranean-type ecosystems, and virtually nothing on plant defoliation by herbivores. Published research includes two papers on shrub defoliation by insects in Chile (Fuentes et al. 1981, Fuentes & Etchegaray 1983), and a review of sclerophyllous Australian eucalypts eaten by chrysomelid beetles (Morrow 1983 based on Morrow & Fox 1980). Some published agricultural research on livestock and range management is indirectly pertinent (e.g. Benjamin et al.1977, Godron et al. 1981).

This thesis focuses on plant-herbivore relationships in three mediterranean-type habitats occurring in California, France and South Africa. These three areas have some overall similarities yet differ in certain critical characteristics. Sclerophylly as a leaf morphological characteristic occurs in many plant species in these ecosystems. The term was first suggested by Schimper(1903) to describe leaves in the mediterranean systems which he had visited and studied. California and France have strong affinities both edaphically and botanically (Di Castri 1981). Soil phosphorous levels are approximately similar, while nitrogen levels are much lower in California. Quercus spp. occur in both ecosystems and in France they are especially widespread. In contrast, the soils in South Africa have extremely low levels of nitrogen and phosphorous and the vegetation is a part of a separate floral kingdom (Capensis) (Werger 1978). The mediterranean systems in Australia are probably the most similar to those in South Africa (Di Castri 1981).

This section has outlined some similarities and differences among the five mediterranean ecosystems and briefly reviewed some published research in specific topics. Herbivory, in particular, has received little attention.

Table 4.0.1 A selection of published values for Nitrogen (N), phosphorous (P) and carbon (C) content of the soils in all five mediterranean regions.

	N %	P %	C %	Reference
SOUTH AFRICA				
Coastal fynbos	0.020 - 0.030	0.002 - 0.003	0.58 - 1.74[1]	Read and Mitchell 1983
Strandveld	0.012 - 0.014	0.008 - 0.010	0.16 - 0.42[1]	This study (Table 5.0.1)
FRANCE				
Garrigue	0.320 - 0.210		3.29 - 2.36	Rapp and Lossaint 1981
"	1.860 - 0.610		21.40 - 4.90	"
"	0.443	0.035		Specht 1981
CALIFORNIA				
Chamise chaparral	0.129	0.054		Specht 1981
Adenostoma	0.040		2.27[1]	Read and Mitchell 1983
Ceanothus greggii	0.080		2.33[1]	"
Salvia spp.	0.180		5.23[1]	"
Eriogonum	0.100		3.43[1]	"
AUSTRALIA				
Mallee	0.042	0.007		Specht 1981
Savanna woodland	0.115 - 0.139	0.022 - 0.030		"
Heath forest/mallee	0.064 - 0.084	0.006 - 0.008		"
Mallee(brown soils)	0.057 - 0.065	0.008 - 0.010		"
Mallee(red/grey soil)	0.045 - 0.110	0.020		"
Banksia/Xanthorrhoea /Casuarina	0.020	0.001	0.12 - 0.93[1]	"
CHILE				
Trevoa shrubs	0.140	0.070	1.86[1]	Adapted from Rundel 1983

[1]Calculated from C % = Organic matter / 1.72 (Specht 1981)

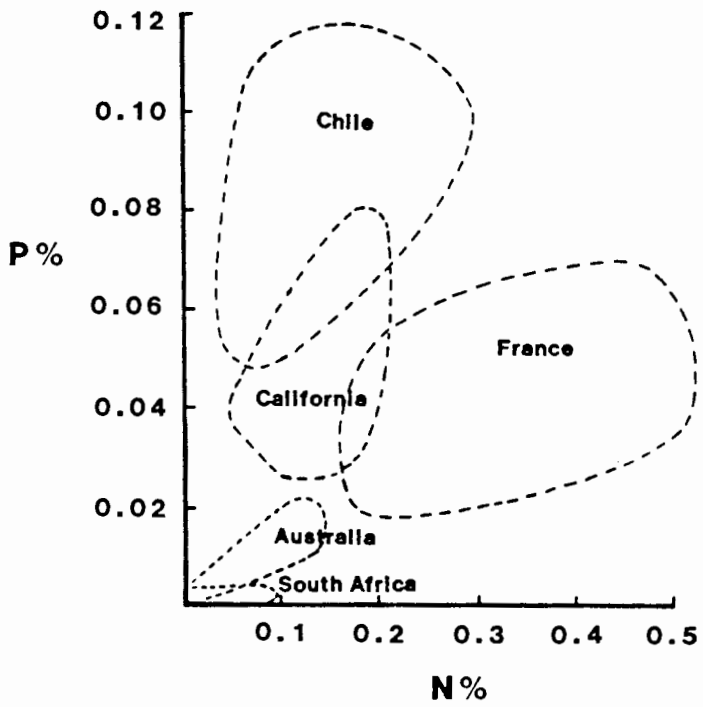


Figure 4.0.1 Nitrogen (%) and phosphorus (%) in the soils of the five mediterranean climate regions (from Di Castri 1981).

5.0 South African strandveld and the antelope study site

The Cape Floral Kingdom covers the smallest land area of the six floral kingdoms (Good 1974, Mooney & Di Castri 1973, Cody & Mooney 1978). The Cape mediterranean-type ecosystems correspond with Werger's (1978) "Capensis" phytogeographical region. The highest number of plant species and of endemics occur here (Goldblatt 1979), but faunal diversity is particularly low (Bigalke 1979).

In this section there is a brief description of South African mediterranean ecosystems and specifically of the South African shrubland known as Strandveld. This is followed by some soil and rainfall data relevant to the strandveld site at which the antelope study was conducted (Chapters 5.1 - 5.4).

The South African mediterranean vegetation has been separated into three distinct agro-ecological types as well as two transitional types (Acocks 1975). The most familiar component of Capensis is fynbos, which corresponds to heathlands as defined by Specht (1979). Fynbos is found on strongly leached, low-nutrient soils derived mostly from sandstone (Specht & Moll 1983). Dominant and ubiquitous families include Proteaceae, Ericaceae and Restionaceae (Kruger 1979). The non-heathland Capensis communities, referred to by Boucher and Moll (1981) as "Shrublands" for reasons of standardization, occur on moderately leached , more fertile soils derived mostly from shale, limestone and granite. Heathlands cover approximately 65 % and shrublands 32 % of the land area of Capensis. The boundaries between shrubland and heathland are unclear: communities form mixed mosaics and there is also some ecotonal interdigitation. Acocks (1975) solved this boundary problem by calling such communities transitional. One of these he named west coast strandveld (Veld type 34),

and the remainder of this introduction as well as the four papers which follow all deal with the vegetation in the South African mediterranean shrubland called strandveld.

Strandveld is the smallest of Acocks veld types (Acocks 1975) covering approximately 600 km^2(Kruger 1979) or 8 % of Capensis land area (Boucher & Moll 1981). It occurs on coastal lowland sands and very little natural vegetation remains amongst large areas under cultivation (Boucher & Moll 1981). This shrubland is analogous to garrigue and maquis in southern France, chaparral in California and matorral in Chile and Spain (Di Castri 1981). Some general structural features of strandveld include: vertical differentiation into usually two canopy layers, although there are occasionally three or four, and a continous canopy horizontally in the absence of disturbance, grazing or fire. A more detailed description can be found in Boucher and Moll (1981).

Strandveld occurs in a true mediterranean climate with some modification of extremes because of the adjacent sea. Annual precipitation is 400 to 500 mm in rainfall with additional moisture from coastal fog, although there is much local microclimate variation. Midsummer daily maximum temperature is about 28° C and declines to 17° C in midwinter; midsummer daily minimum is about 15° C and 6° C in midwinter. Air temperature can reach as high as 47° C in summer (Boucher & Moll 1981) and exposed soil surface temperatures even higher than this.

The field work for the research reported in Section 5 was conducted on "Rondeberg" farm (33°25' S, 18°16' E) on the Atlantic ocean coast 65 km north of Cape Town. The farm covers approximately 2000 ha of strandveld with the ocean and the Cape Town-Saldanha road forming parallel boundaries. The study site was located more than 2 km from the main road in relatively homogeneous stable vegetation (Figure 5.0.1).

Temperature and rainfall profiles relevant to the study site were obtained from two weather stations close to Rondeberg. The data from these two stations were similar to each other and to the maxima and minima mentioned above for all strandveld areas. Precipitation, however, can vary locally and rainfall was recorded for the duration of the study because of this. This monthly record, the rainfall record from the two nearby weather stations and a water-table record are shown in Figure 5.0.2. Fluctuations in the water table were monitored using a plumb-line dropped into a disused well. Between 73 and 75 % of total annual rainfall occurs between May and September: this pattern is reflected in the fluctuations of the water table.

There appear to be seven major soil types on which shrublands occur. Strandveld seems to be found mainly on acid arenosols, solonetzic and planosolic soils. The arenosols are yellow to white aeolian sands with low silt to clay ratio while the other two soils have higher fertility and are derived from shales and mudstones (Boucher & Moll 1981). The soil on the study site was predominantly a yellow aeolian sand overlaying a granite shelf. The presence of numerous seasonal springs on the study site suggested that ground water was trapped above this granite shelf. For phosphorus determination, four samples were collected on the soil surface and four more from 5 cm below the surface. For nitrogen determination, four soil samples were collected more than 2 m from the nearest plant and four samples were collected adjacent to Willdenowia plants. These results (Table 5.0.1) suggest that N levels in Strandveld fall within the range for Capensis heathland (Boucher & Moll 1981) rather than among the higher levels characteristic of shrubland (Table 5.0.2). The value for coastal fynbos, a heathland, is anomalously lower than that for strandveld. Strandveld phosphorus levels fall well within the range of Capensis shrubland which is higher than the range for Capensis

heathland. Once again, the value of Specht and Moll (1983) for coastal fynbos is anomalously higher than the strandveld value, and also above the Capensis heathland range. Strandveld therefore does not seem to resemble closely its adjacent vegetation counterpart , coastal fynbos, although it does fall within the N and P ranges of both shrubland and heathland. This perhaps is a partial explanation of strandveld's transitional vegetational characteristics.

The vegetation on the study site conformed to the general description mentioned above (Boucher & Moll 1981). Grazing activity of antelope produced a network of narrow paths which dissected the canopy into larger and smaller clumps. These clumps contained woody, sclerophyllous species such as Colpoon compressum, Euclea racemosa, Olea exasperata and Pterocelastrus tricuspidatus as the tallest (2.0 - 2.5 m) components. Shorter sclerophyll shrub species encircled the tallest elements and included Tetragonia spp, Rhus incana and R. lucida, Zygophyllum morgsana and Putterlickia pyracantha, as well as succulents such as Cotyledon orbiculata and Euphorbia spp. Below this was a layer which included species such as Antizoma capensis, Aspalathus hispida, Helichrysum spp, and very widespread Nylandtia spinosa. Grasses, which were present depending on season, included Ehrharta spp. and Ficinia dunensis. This vegetation also contained a second type of often smaller clumps composed of Willdenowia striata (Restionaceae) and/or Thamnochortus spp. along with Ehrharta spp. and Ficinia dunensis and a sclerophyllous shrub Eriocephalus racemosus. The most noticeable seasonal change apart from phenophase changes in the vegetation was the occurrence in spring of a flush of annuals, most of which were Asteraceae and some Liliaceae.

The Ethiopian Zoogeographic region encompasses the mediterranean ecosystems of

southern Africa, including the macchia shrubland called Strandveld (Darlington 1957, Bigalke 1972, Specht & Moll 1983). Palaeontologically, strandveld and closely related coastal fynbos (macchia) comprise one of three vegetational subdivisions (Acocks 1975) in the Cape biotic zone (Hendey 1974). This zone covers the south-western Cape. Compared with the rest of the Ethiopian region, this area has contained an impoverished mammalian fauna since the late Pleistocene. However, there are fossil remains of some endemic mammals including rodents and antelope. The antelope remains include two neotragines, Raphicerus melanotis (grysbok) and R. campestris (steenbok) (Klein 1975).

Table 5.0.1. Nitrogen[1] and phosphorus[2] levels in the soil at Rondeberg ($\mu g\ g^{-1}$d.w.).

	n	$\bar{x}$	S.D.
Total N	8	136.00	8.00
NO_3^-	8	1.19	0.30
NH_4^+	8	1.46	0.73
Total P	8	87.60	12.10
Available P			
Bray No. 2	8	4.84	1.62
Resin Bag	6	8.33	3.18
% organic matter	8	0.50	0.23

[1] Nitrogen values from W D Stock, unpublished data.

[2] Phosphorus values from G Brown, unpublished data.

Table 5.0.2. Soil nitrogen and phosphorous levels for some South African mediterranean vegetation sub-types.

	% N	% P	Reference
Strandveld	0.0136	0.0088	This study(Table 5.0.1)
Capensis heathland	< 0.0500-0.1000	< 0.0020-0.0500	Boucher & Moll 1981
Capensis shrubland	0.0500-1.0000	> 0.0050	"
Capensis forest	>1.0000	> 0.0030	"
Renosterveld	0.0400-0.5000	0.0075-0.0150	Specht & Moll 1983
Mountain fynbos	0.0400-0.1000	0.0027-0.0015	"
Coastal fynbos	0.0050	0.0600	"
Coastal fynbos	0.2600	0.0044	Kruger 1979
Mountain fynbos	0.1000-0.2100	0.0001-0.0038	"

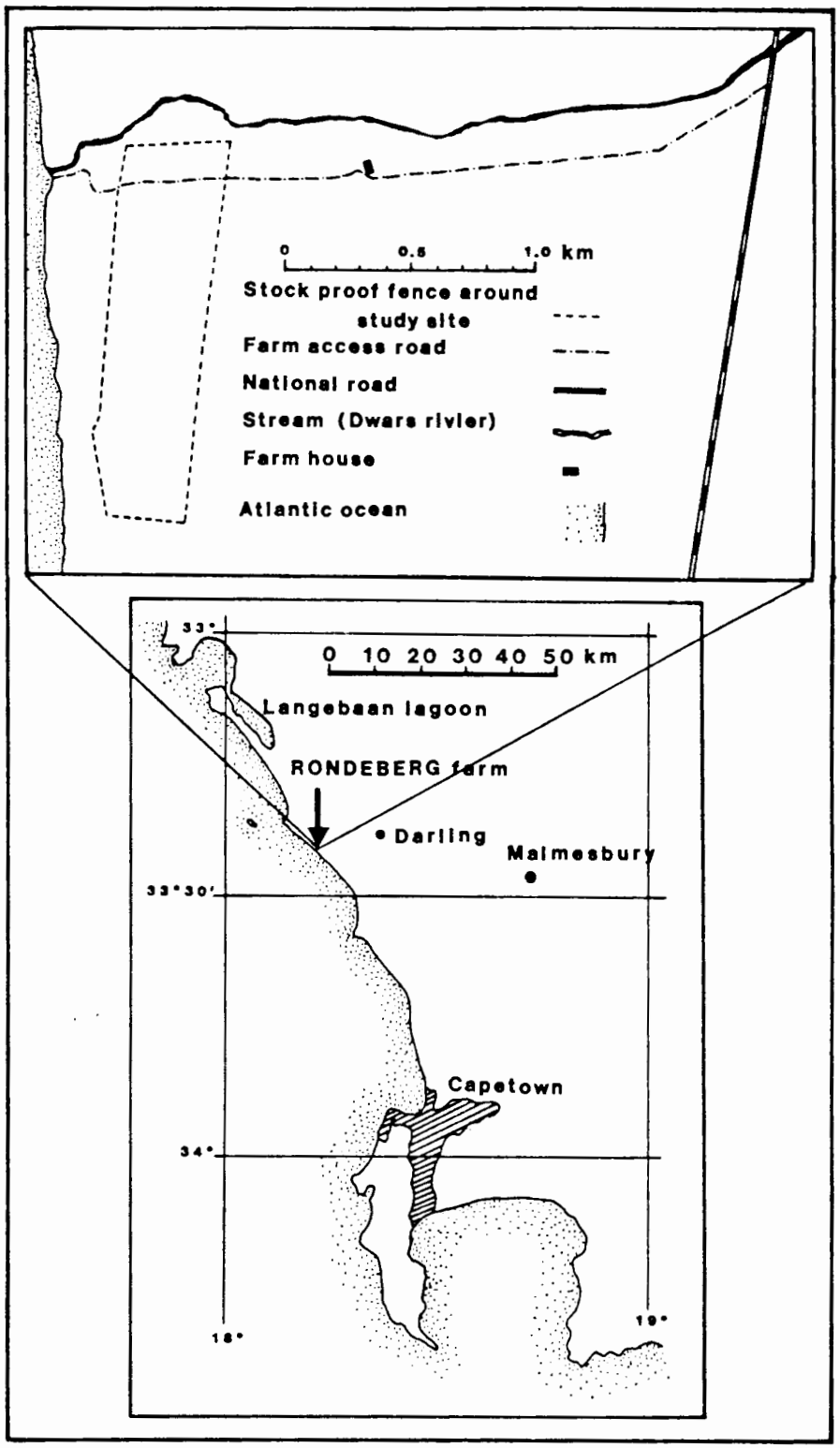


Figure 5.0.1 The location of the study site, Rondeberg Farm, north of Cape Town, South Africa.

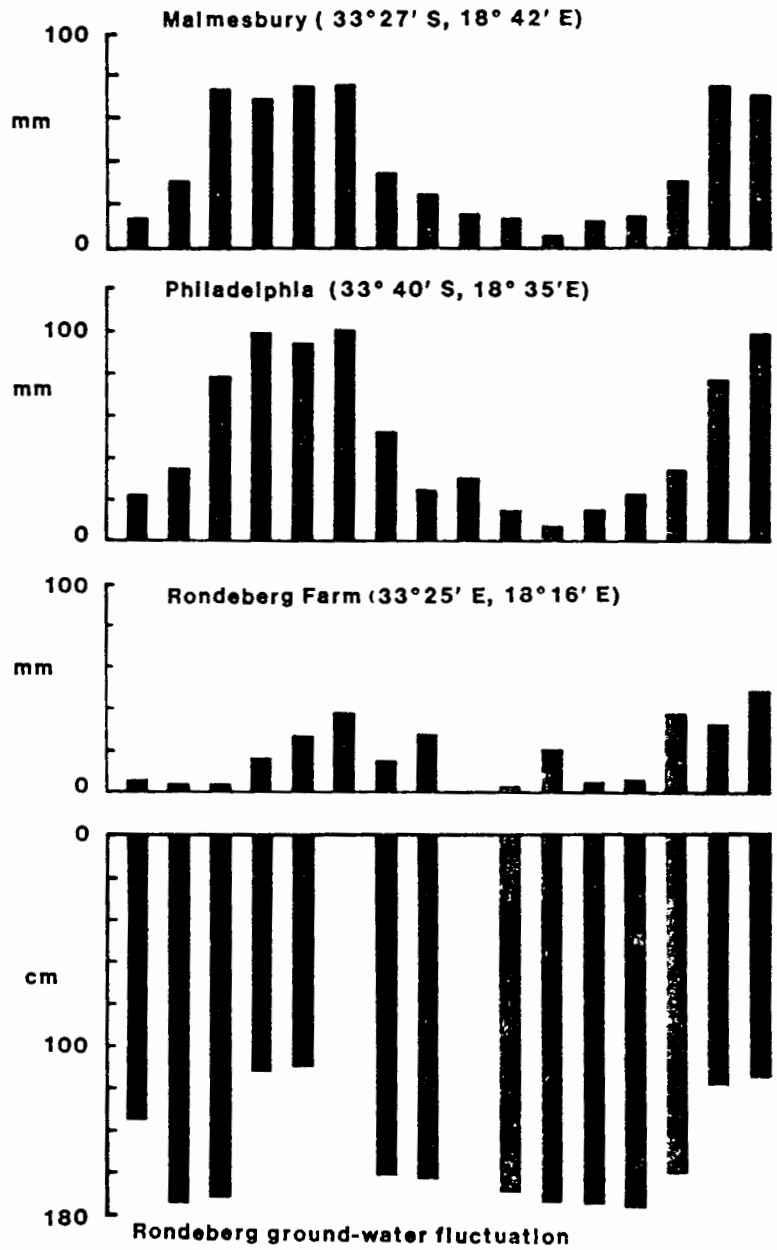


Figure 5.0.2 Monthly rainfall (mm) at Rondeberg farm and two adjacent weather stations, and fluctuations in groundwater levels at Rondeberg, for the duration of the study. The weather station data was provided by the Agricultural Meteorologist, "Bien Donne", Groot Drakenstein, Cape.

5.1 BROWSE SELECTION OF SMALL ANTELOPE IN A SOUTH AFRICAN MEDITERRANEAN SHRUBLAND

5.1 INTRODUCTION

Much literature about grazers and grasslands in tropical and sub-tropical Africa exists (Field 1976). Very little has been published about browsing (except for Owen-Smith 1979, Van Hoven 1984) and almost nothing quantitative has been published about antelope browsing and grazing in South African mediterranean heathlands and shrublands, apart from two theses (Manson 1974, Norton 1980).

The Cape grysbok R. melanotis is largely restricted to the mediterranean vegetation called Fynbos (Manson 1974, Bigalke 1979) while R. campestris (steenbok) and a third Cephalotragine antelope, Sylvicapra grimmia (common duiker), are widespread in sub-Saharan Africa (Dorst and Dandelot 1970). All three species are small(7 - 18 kg) and solitary (Dorst and Dandelot 1970). They would be classified as selective browsers (Wilson 1966, Manson 1974) according to observations of their plant feeding preferences. However, based on rumen structure and capacity, Hofman and Stewart (1972) would classify them as intermediate eaters since all, but particularly R. campestris, also consume grass.

In the ecological literature on animal feeding, there appear to be two major theoretical approaches to feeding studies: optimal foraging theory on the one hand and preference studies on the other. Preference studies aim to determine some ranking of food items as accurately as possible without explaining how a given animal arrives at these choices. In most optimal foraging studies an assumption is made that net energy intake per unit time is being optimised and that any ranking of food items can be explained on this basis. Large differences in nutritional value exist both among different plant species (Waterman et al. 1983) and among different plant parts (Feeny 1970, Maynard &

Loosli 1969, Robbins 1983). Seasonal phenophase changes make these differences variable in time. Because of this, a mobile herbivore needs to make necessary adjustments in selection of food items in accordance with its own metabolic requirements which may fluctuate seasonally. Small ruminants, while having proportionally smaller gut capacities, have higher relative or mass specific metabolic maintenance requirements and limits to retention times (Van Soest 1983, Janis 1976). In order to achieve intake of all necessary nutrients, selection needs both to be diverse as well as to maximize protein and minimize fibre intake (Van Soest 1983, Milton 1979). Optimal foraging theory (Pyke et al. 1977, Pyke 1984) has been useful to some extent as a theoretical basis for exploring this problem(Emlen 1966, Schoener 1971, Westoby 1974 but see Westoby 1978, Belovsky 1978). However, a number of studies have questioned both the validity of using net energy intake per unit feeding time as the single factor being optimised and the ability of the animals to be able to rank items in a specific way. These include work on the ecology of protozoa, insects, mammals, and birds feeding on marine and terrestrial invertebrates (Schluter 1981, Rapport 1981, Zach & Smith 1981). This approach to foraging comprises more of a cost : benefit analysis than the more descriptive studies where attention has been paid directly to preference. The latter studies have a longer history in the fields of agriculture and range management, and as such have had to contend directly with the practical problems of sampling and of large variance in animal behaviour.

In preference studies, Petrides (1975) distinguished between principal foods (those eaten in greatest quantities) and preferred foods (those proportionally more frequent in the diet than in the environment). He discussed the usefulness and applicability of several food preference indices, including that of Ivlev (1961). Barnes (1976) suggested that dietary preferences can be estimated using four types of methods: 1) measurement of vegetation

being grazed and/or browsed, 2) direct observation of the animal, 3) analysis of ingesta or faeces, and 4) offering plant material on a free choice basis ("cafeteria tests") and measuring relative consumption. Plant -based techniques for measuring browse have been reviewed by Rutherford (1979). In this study I investigated browse patterns of small antelope in strandveld vegetation using plant-based techniques and expressing antelope preferences in terms of Jacobs' D selectivity index (Jacobs 1974). I also used direct animal observation to check the validity of the results obtained in this way.

5.1 METHODS

Vegetation sampling

The vegetation was sampled to quantify both the species composition and the evidence of antelope browse a) by a plot-less sampling method using random points and b) by recording species occurrence and evidence of browse along trails used by antelope in the vegetation. Sampling was conducted in an eight ha area of homogeneous strandveld (Acocks 1975 -veld type 34) which was part of a 65 ha camp from which livestock had been excluded for more than six months prior to sampling.

Random point transects

A set of random tables (Zar 1974) was used to select a set of 100 random intervals along a 50m length. These were cumulatively added together and recorded on a field sheet. A grid was superimposed on a aerial photograph of the study area and 10 randomly chosen coordinates were located on the photograph. A 50m tape-measure was then

staked out in the field, through each coordinate in turn. Each of the 100 random points was placed in sequence along the tape. At each point, the presence of sand, woody biomass, litter or plant species was noted as it intercepted the cross-hairs of a gimballed sight viewed vertically from above (Warren 1965, Griffiths & Barker 1966). The presence of antelope-browsed leaves or twigs was also noted. The use of this sighting device removed any observer bias and provided a rapidly repeatable plot-less sampling method which ensured that "plant size" effects were rendered insignificant (i.e. larger plants were not more likely to be chosen than small plants purely because of size). One thousand random point observations (10 transects x 100 points on each) were collected monthly in September 1979 and December 1979 - June 1980. It was possible to monitor seasonal changes in the vegetation using this random point transect data. The different plant species were categorized into five life-form groups according to obvious differences: broad-leaved shrubs including thorny species; fine-leaved shrubs including thorny species; herbs, forbs, grasses, annuals and creepers (grasses and forbs henceforth); succulents; and Restionaceae . Restionaceae were separated from grasses because they are perennial, very abundant and endemic in this shrubland.

Trail transects

Animal trails were common between the strandveld bush and Restionaceae clumps, and some of these were followed as transects usually for distances of approximately 0.75 km. A given transect was only sampled once in order to sample as wide an area as possible. Evidence of browse and the phenophase status of each individual plant along both sides of sections of these trails were categorized qualitatively and recorded on a field sheet format adapted from Walker (1976). The seven phenophase categories are listed in Figure 5.1.3; however in Appendix 5.1.2 an additional category has been

included (0 = pregrowth) which, in Figure 5.1.3, was combined with category 7. An average of 320 plants in 70 species were examined monthly from December 1979 - June 1980. It was possible to check on criteria for establishing the "age" of browse evidence by examining the vegetation in the captive antelope enclosures (see below) during the course of that experiment. New browse was quite distinctive: the newly exposed plant tissue was usually very pale and would darken as cells and exudate became oxidised, sometimes as rapidly as a couple of hours. This sampling method provided information on plant species availability and browse patterns for summer, autumn and winter, also thereby encompassing both dry and wet seasons.

Direct feeding observations on antelope

In order to check the results generated from the plant-based methods outlined above, a field experiment was conducted during April 1980. Two captive steenbok R. campestris and two grysbok R. melanotis were obtained from Tygerberg Zoo (40 km from Cape Town) where they had been reared. These four small antelope were released into a 400 m^2 net enclosure in an adjacent area to that in which the transect study was being conducted, and their feeding preferences were recorded. The enclosure was moved every seven days to prevent over-utilization of the vegetation and to minimise the possible complicating effects of local heterogeneity in the vegetation. The vegetation in each enclosure was mapped using a 2m x 2m grid to determine cover. Relative abundance of each species was measured by recording the total number of individual plants in each species. The animals were weighed before and after the trials to monitor any change in condition. Over a period of seven weeks there was an average weight loss of 4.5%.

Data analysis

Data from trail and random point transects were compared using a simple linear regression correlational analysis. Browse indices (see below) were calculated using both the trail and random point transect data and the results of the field feeding experiment.

The browse data for each plant species from the trail transects was transformed into a selectivity index, Jacobs (1974) D index:

$$D_i = \frac{r + p}{r + p - 2rp} + 1$$

where $r = \frac{\text{ratio of species i browsed}}{\text{total number of browse observations}}$

$p = \frac{\text{ratio of species i available}}{\text{total number of species available}}$

Sets of D indices for individual plant species were calculated from browse observations which were grouped in turn into separate seasons (summer, autumn and winter) and also into dry and wet seasons. This study was primarily concerned with the perennial woody species and the logistics of gathering data on the spring flush of annual plants were considered to be beyond the scope of this project.

The degree of new browse on individual plants on the trail transects was categorised and recorded according to a simple scheme:

1 = < one-third of foliage

2 = one-third to two-thirds foliage

3 = > two-thirds.

To simplify analysis, an individual plant which, for example, had been heavily browsed (more than two-thirds) was recorded as though a total of three individual plants had each been browsed lightly (i.e. 1= < one third of foliage with browse evidence). This would then raise the browse frequency (r) accordingly for that particular plant species, since no adjustment was made in the frequency of occurrence (p). Scoring browse in this way removed any necessity for a system of "weighting" with its attendant complications. Jacobs D indices were also calculated for the results from the captive antelope feeding experiment.

5.1 RESULTS

Spatial and temporal changes in the vegetation

Approximately 50% of random points intercepted photosynthetic material. The remainder of the points intercepted sand and litter (29.7 ± 5.3*% in the dry season; 25.1 ± 2.0*% in the wet season) and woody material (20.1 ± 3.3*% in the dry season; 16.7 ± 2.0*% in the wet season). The plant species intercepted in the photosynthetic material category were identified (Appendix 5.1.1) and grouped together in five life form types (Figure 5.1.1). There were some minor increases and decreases in vegetation abundance when comparing wet and dry seasons (Figure 5.1.1). However, most noticeable was the increase in the abundance of forbs and grasses with increased precipitation.

As expected, phenophase changes in the vegetation during the transition from the driest period to the wet period were more obvious than any differences in relative abundance of particular life forms (Figure 5.1.2); evidence of flowering and fruiting are unmistakeable. These data suggest that flowering, at least, began towards the end of summer before the wet season. Fruit then developed during the wet season coincident with the period when soil nutrients are most mobile and readily absorbed by plants. Fruiting appears to have been completed by January. The trend in relative abundance of new growth suggests that all plants were growing new tissue during summer, albeit very slowly. The highest occurrence of new growth in April, May and June is partially attributable to the production of flower buds which are not always distinguishable initially from other kinds of new tissue.

* STANDARD DEVIATION OF THE MEAN

The phenophase changes in each of the five major life form categories (Figure 5.1.3) provide more detail about this generalised picture (Figure 5.1.2). Specifically, fine-leaved plants were fruiting and post-fruiting in December through March. In February, this group showed increased evidence of new growth followed in April by buds and flowers. Broad-leaved plants showed most new growth in mid-summer followed by a steady decline through to the wet season. Numbers of flowering plants were fairly constant from month to month, while fruiting was constant in January and March. The pattern in succulents clearly suggests that plants were post-fruiting in December and became senescent thereafter until the precipitation increased and all plants rapidly turned to new growth. Half the Restionaceae appeared to be post-fruiting or senescent in December while half showed evidence of new growth. In January some plants were flowering and some fruiting. During March most were actively growing followed in April by flowering, and by ripe fruits (seeds) in May and June. Finally, forbs and grasses were very sparse until April and the following months when there was a rapid increase in their relative abundance. Phenophase diagrams for nine species are given in Appendix 5.1.2. These major spatial and temporal differences in plant life-form types, each with its different phenophase, provided a wide spectrum of possible dietary components for small antelope.

Browse and plant selectivity

The frequency of browse in the vegetation followed the same general pattern from month to month during the period of observation (Figure 5.1.4): it declined to a minimum in March or April. The random point method yielded frequencies which were approximately 3 to 8 times lower than those for trail transects. This supports the observation that these trails were used for foraging by antelope.

Although there was a difference in the overall frequency of browse in the vegetation as measured by these two methods, the significant correlation coefficient of species abundances in the whole vegetation (Figure 5.1.5) in both wet and dry seasons suggests that there was satisfactory agreement between the two methods in obtaining a measure of abundance. Likewise, browse frequency for each plant species was found to correlate significantly with both random point and trail transects (Figure 5.1.6).

Plant species selectivity was determined on the basis of life forms (Figure 5.1.7) and individual species (Table 5.1.1). During summer, both fine-leaved and broad-leaved plant species were eaten at least as frequently as they were abundant, while fine-leaved plants appeared to be preferred. In March and April, a shift occurred to succulents, grasses and forbs. Grasses and forbs were preferred while succulents were eaten in proportion to their abundance. Finally, Restionaceae appear to have been avoided although during May there was an increase in the use of this family. During this period, these plants produced nut-like seeds which seem to have been a preferred item.

Jacobs D indices of selectivity were available for 24 plant species during both wet and dry seasons (Table 5.1.1). The lower frequency of occurrence of browse on random point transects resulted in a higher number of zeroes which, according to the formula for Jacobs D indicates complete avoidance of those particular species. As expected, the higher frequency of browse on trail transects resulted in fewer zero indices. Monthly selectivity diagrams for 19 of these species as well as four other species are given in Appendix 5.1.3.

Feeding trial using captive antelope

The composition of the vegetation in the site where this field experiment was conducted was compared with the composition of the vegetation in the larger browse study, even though they were only 0.25 km apart. The ground cover area (arcsin transformed) for each plant species present in feeding trial enclosures was significantly correlated with the percentage frequency of occurrence (arcsin transformed) for both the random point transects ($r = 0.87$, $df = 30$, $p < 0.001$, $y = 1.23x - 3.27$) and trail transects ($r = 0.57$, $df = 30$, $p < 0.001$, $y = 1.08x - 2.11$). This indicates that plant species abundances of the two sites were sufficiently similar to permit comparison of the browse data obtained from each site.

The captive antelope showed a wide range of selectivity and consequently there was no significant relationship between the abundance of plant species available (expressed as ground cover area) and the mean time spent feeding on a given species (Figure 5.1.8). These data were then used to derive Jacobs D indices for each plant species and the resulting indices were compared with indices derived from the random point and trail transects (Table 5.1.1). The indices obtained in the feeding trial showed significant linear regressions both with the indices from the random points ($r = 0.48$, $df = 22$, $p < 0.02$, $y = 0.51x + 0.77$) and with the indices from the trail transects ($r = 0.44$, $df = 22$, $p < 0.05$, $y = 0.54x + 0.71$). I interpreted this as experimental support for the data obtained from the vegetational evidence.

Finally, a comparison of the numbers of plants within the same 15 plant species with a Jacobs D score of 0, less than 1, 1 or more than 1 in the random point transects, trail transects and captive antelope feeding trial (Table 5.1.2) suggests that the captive animals

were much less selective than their free-ranging counterparts. They consumed all 15 plant species to some extent in contrast with the evidence from trail transect and random point transect indices where one and four zeroes respectively were recorded. Much more noticeable was that these captive individuals preferred (Jacobs D indices > 1) double the number of plant species, which suggests that overall they were less discriminatory than wild, free -ranging antelope.

5.1 DISCUSSION

Plant phenology and browse

One definitive feature of mediterranean climate is the clear seasonal division based on precipitation into long, dry summers and cool, wet winters. The results of this study have shown the degree to which plant species abundances change and how these, together with phenophase cycles, affected browse selectivity of small antelope.

As expected, minor changes occurred in abundances of plant species, an exception being the rapid increase in grasses and forbs with increased precipitation in autumn/early winter. Comparison of the phenophases of five major life-form categories showed that these groups of species were in different stages of their growth and development cycles at any one time. Fine-leaved plants shifted from a post-fruiting to a new growth phase from December to June. Fruit disappeared after February and flowers became more abundant as June approached. Browse evidence was highest from December to March but declined through to June and the D indices declined below 1, indicating avoidance of these plants. Broad-leaved species appeared to have most new growth in mid-summer with a steady decline through to winter. Flowering occurred more or less constantly, i.e. at least some broad-leaved species were flowering at all times. Nevertheless, fruiting was higher in January and March. Browse indices on these plants peaked in February and then declined below 1 but not below a value of 0.4. Succulent species were almost post-fruiting in mid-summer followed by senescence and then a gradual increase in new growth during early winter. Browse indices suggested that they were avoided during December to March but with new growth they were eaten in proportion to their abundance. Restionaceae showed no clear phenophase pattern except that by

March all individuals showed signs of new growth, in April there was some flowering and in May and June some fruiting. Antelope largely avoided browsing them until early winter when there was much evidence that they consumed the seeds (personal observation). During the dry mid-summer, grasses and forbs were, as expected, mostly absent. With increased precipitation in April and May, there was a burst of new growth and these plants were heavily browsed. In summary, it appears that browse mostly occurs in those groups of species or life forms which are producing new vegetative growth or in some cases are fruiting. There was evidence that six species were completely avoided during the wet season and three in the dry season. These were Colpoon compressum, Phylica stipularis, Agathosma imbricata, Salvia aurea/nivea, Zygophyllum flexuosum/morgsana, and Cynanchum sp. in the wet season and Euclea racemosa, Agathosma imbricata and Amphithalia sp. in the dry season.

As part of a larger general study of R. melanotis (grysbok), Manson (1974) examined the gut contents of 16 animals and compared them with those of four R. campestris and three S. grimmia. The data were limited for comparative purposes because they represent a single sample of plant material in time. However, 17 of the 20 plant species identified (> 70%) were also browsed by the same antelope species in this study.

Random point and trail transects

Random point transects proved to be an effective method of gathering data both about vegetation composition and the frequency of browse on individual plant species. However, as shown in comparison with the trail transects (Figure 5.1.4), the sample size of random points would have had to have been four times larger to have obtained similar estimates of browse. One disadvantage of the trail transects was that, because of

the physiognomic structure of the vegetation, larger, woodier species tended to be in the middle of bush clump islands and tended therefore to be undersampled by this method. However, small and seemingly insignificant plants which may have been important dietary constituents were taken into account. An ideal improvement for both methods would have been the use of predictive regression equations to estimate the plant biomass removed at a damaged point (Shafer 1963, Schuster 1965, Basile and Hutchings 1966, Barnes et al. 1976). This would have meant deriving a set of equations for each plant species being studied, and the probable advantages would have been offset by an enormous amount of very painstaking field work.

Jacobs D index (1974) is a significant improvement over Ivlev's electivity index (1961). It is particularly suitable for studies such as this. Westoby (1974) and Petrides (1975) point out some of the disadvantages of Ivlev's index, and these are removed in the D index. The major one for the electivity index is that an abundant and a scarce resource may both have the same index value because they have been used in proportion to their abundance. D is independent of relative abundance of food available and Chesson (1978) has shown that a similar measure suggested by Manly et al. (1972), also like D and others based on Ivlev's index, can be rigorously derived from a stochastic model. This makes them easily interpretable and useful in a wide range of circumstances.

Antelope feeding trial

The findings from the captive antelope experiment supported those from the larger browse study. Numerous published studies have shown that ecological experiments can greatly enhance descriptive and observational studies. Nevertheless, the results highlight some of the pitfalls of using animals which have not been reared in the

experimental environment. The captive antelope appeared to be much less discriminating about their choice of plant species than the wild antelope were, according to the evidence of browse recorded in this study. Sheep in pasture grass palatability tests were influenced by pre-conditioning (Marten 1978), while small differences in early experience on one type of natural grazing resulted in marked differences in grazing preferences much later (Arnold & Maller 1977). This effect was also noted in young antelope (Leuthold 1971).

A number of methodological factors introduced difficulties in interpreting and extrapolating from these results . These included small sample size of antelope (n = 2 for each species) , estimation of biomass consumed, and restriction of the animals to an unnaturally small range in spite of regular shifting of the enclosure. One interesting finding was a significant correlation between preference index and the calorific value of each species (Spearman rank $r_s = 0.56$, $n = 14$, $p < 0.025$) (N. Adams, personal communication). This suggested that these antelope were able to adjust their intake at least to ensure improved energy gain, perhaps because the enclosure introduced an unnatural degree of restriction.

The relationship between phenophase and browse preference and avoidance is complex, and involves an interplay between plant and animal ecophysiology. The data from this study only permitted an examination of patterns at the community level. A more detailed biochemical/ nutritional description of individual plant species would certainly provide more insight about browse preference and avoidance. The following two chapters cover this topic.

Table 5.1.1. Jacobs D indices(see text for details) for free-ranging and captive antelope browse on 24 shrubland plant species. Indices of 0.00 indicate that a plant species was completely avoided, and indices of 2.00 indicate that a species was highly preferred. (- = index could not be calculated because either browse or abundance data were not measured)

	Wet season		Dry season		Captive
	Random points	Trail transect	Random points	Trail transect	antelope trial
Willdenowia striata	0.54	0.85	0.06	0.17	0.17
Pterocelastrus tricuspidatus	0.00	1.21	0.74	1.06	0.21
Euphorbia caput-medusae/burmannii	1.44	1.08	0.79	0.88	0.84
Nylandtia spinosa	1.43	1.34	1.88	1.53	1.74
Euclea racemosa	0.49	1.00	0.00	0.00	0.67
Asparagus capensis	1.52	0.92	0.00	0.80	1.77
Chrysanthemoides incana	1.35	1.00	1.00	1.27	1.15
Colpoon compressum	0.00	0.00	0.00	1.00	-
Putterlickia pyracantha	0.00	1.12	1.41	1.07	1.34
Rhus incana	1.00	1.00	0.80	1.00	0.95
Senecio elegans	1.62	1.00	0.00	1.20	-
Passerina vulgaris	1.00	1.15	0.00	1.00	-
Phylica stipularis	0.00	0.00	0.66	0.39	1.67
Olea exasperata	-	-	0.00	1.00	-
Ehrharta erecta	1.46	1.27	0.00	1.00	-
Agathosma imbricata	0.00	0.00	0.00	0.00	-
Amphithalia sp.	0.00	1.00	0.00	0.00	-
Thesium aggregatum	0.00	1.34	1.34	1.66	1.96
Trachyandra revoluta	0.66	1.18	0.00	1.00	-
Tetragonia fruticosa	0.39	0.80	1.00	1.00	-
Salvia aurea/nivea	0.00	0.00	1.00	0.66	1.75
Zygophyllum flexosum/morgsana	0.00	0.00	1.00	1.00	-
Cynanchum sp.	0.00	0.00	0.00	1.00	-
Ficinia dunensis	1.00	0.66	-	-	-

Table 5.1.2. Number of plant species browsed from the random point transects, trail transects and captive antelope feeding experiment which scored values of Jacobs D index equal to zero (uneaten), <1.00 (eaten less than available), equal to 1.00 (eaten as frequently as abundant) and > 1.00 (eaten more than available i.e. preferred). Data from Table 5.1.1.

	Vegetation study		
Jacobs D index	Random points	Trail transects	Antelope trial feeding experiment
= 0.00	4	1	-
< 1.00	6	4	6
= 1.00	2	6	-
> 1.00	3	4	9

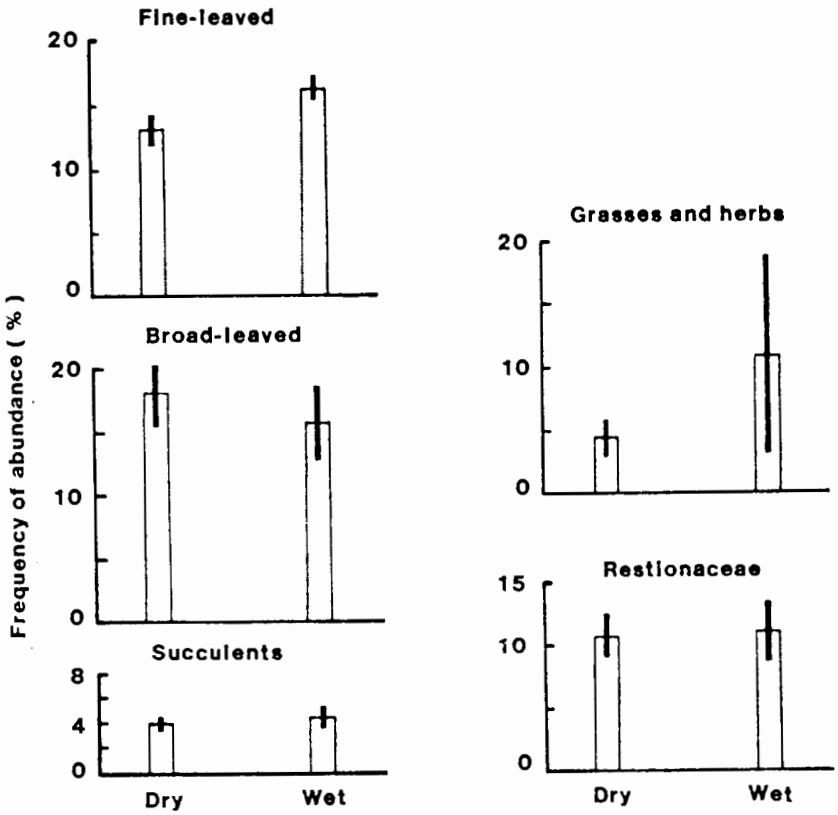


Figure 5.1.1 Vegetation composition (%) of each of five major life forms in dry and wet seasons in Strandveld vegetation. Composition was determined using random point data (see text). Means for the dry season were calculated from 5 months' data and for the wet season from 3 months' data.

Error bars indicate ± 1 standard deviation from the mean.

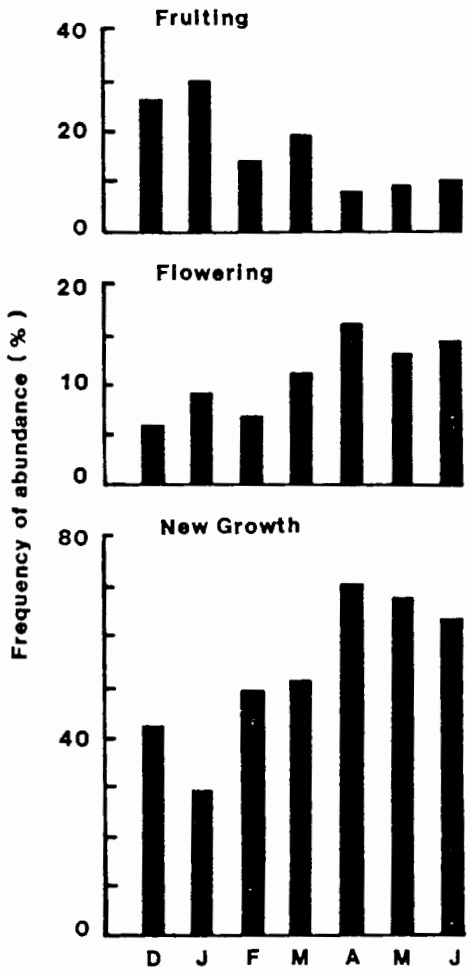


Figure 5.1.2 Whole community phenophases for the Strandveld, derived from trail transect data (see text).

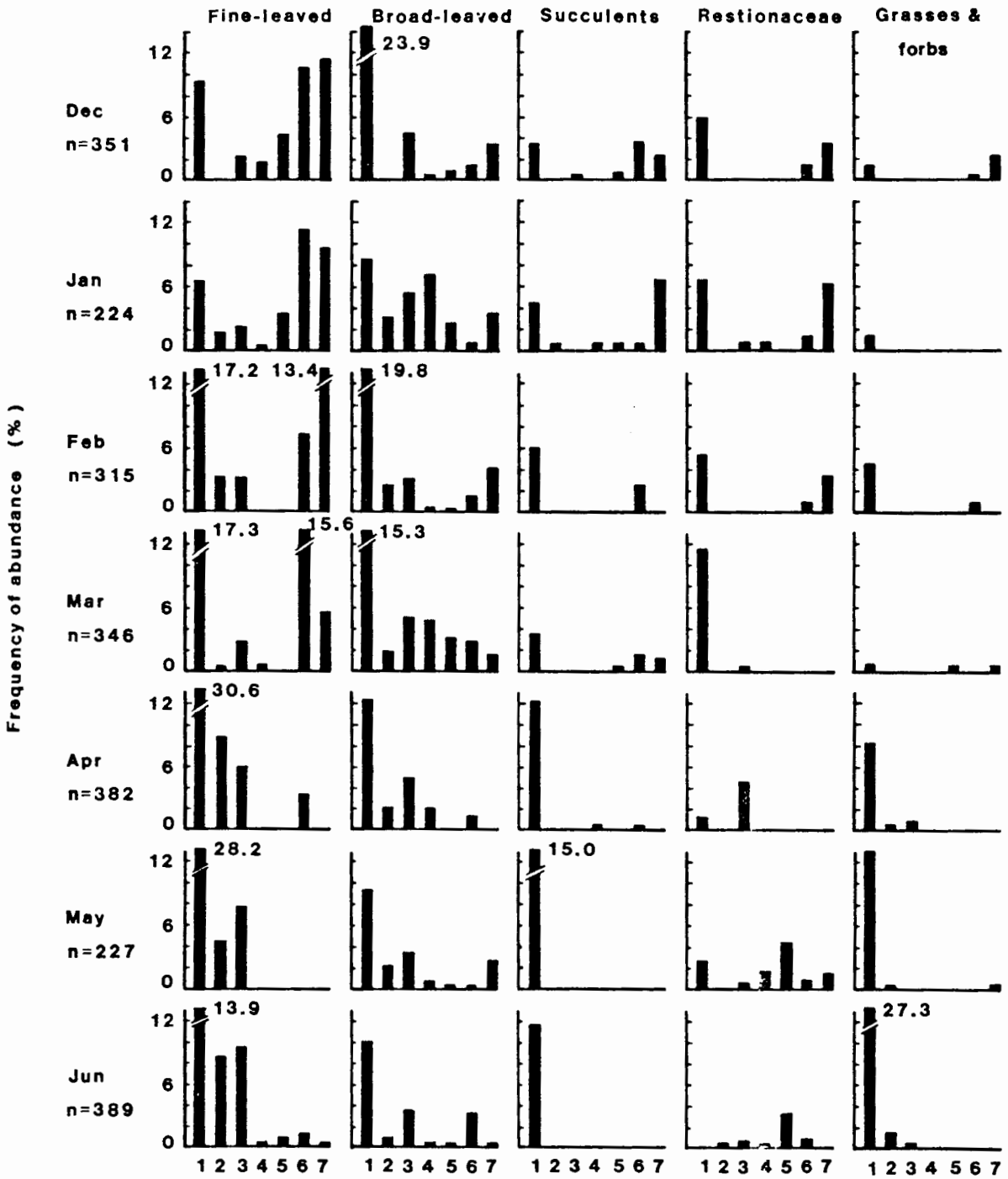


Figure 5.1.3 Phenophases of the major life form categories in Strandveld. Percentages were calculated for each month separately. Sample sizes of the total number of plants examined each month are indicated. Phenophase categories: 1 = new growth, 2 = flower buds present, 3 = flowering, 4 = developing fruit, 5 = ripe fruit, 6 = post-fruiting, 7 = senescent.

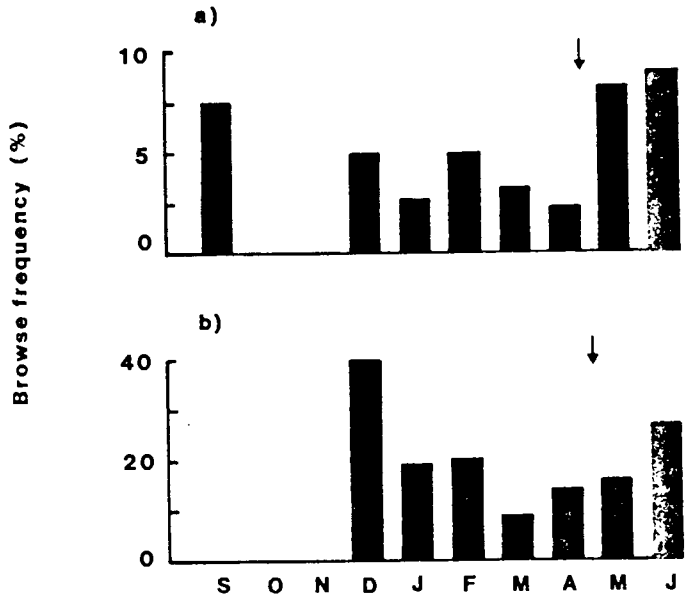


Figure 5.1.4 Browse frequency in Strandveld vegetation derived from a) random point transects and b) trail transects. An arrow indicates the end of the dry season.

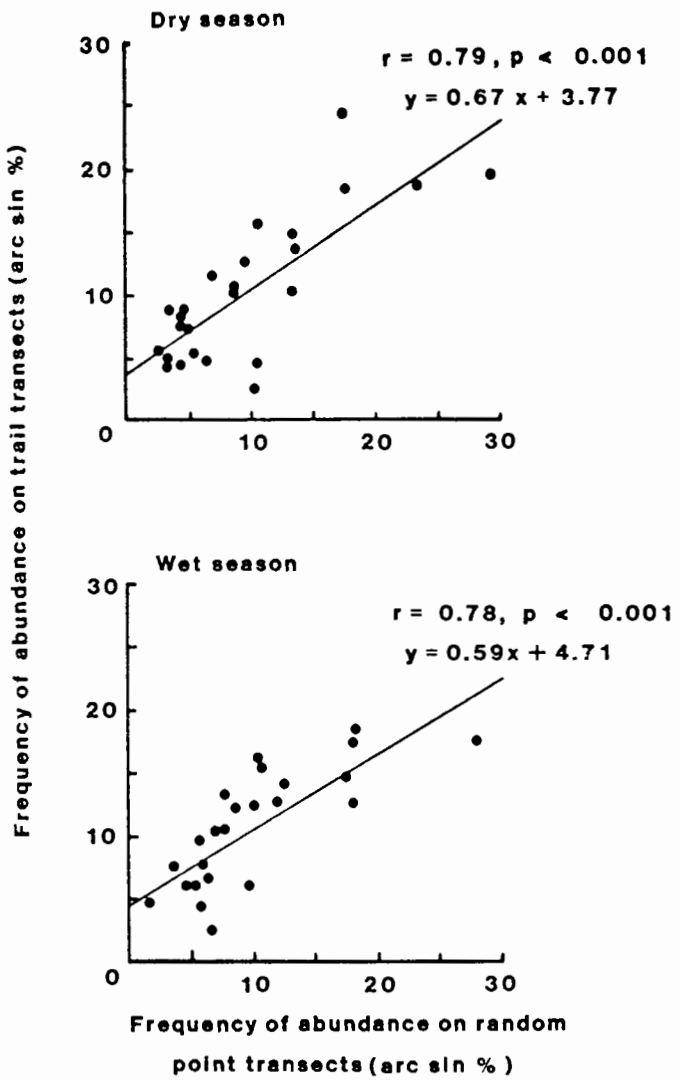


Figure 5.1.5 The relationship between the frequency of occurrence of plant species on antelope trail transects and random point transects in Strandveld vegetation in dry and wet seasons. The frequency of occurrence of each species was expressed as a percentage of all species encountered and these were arcsin transformed.

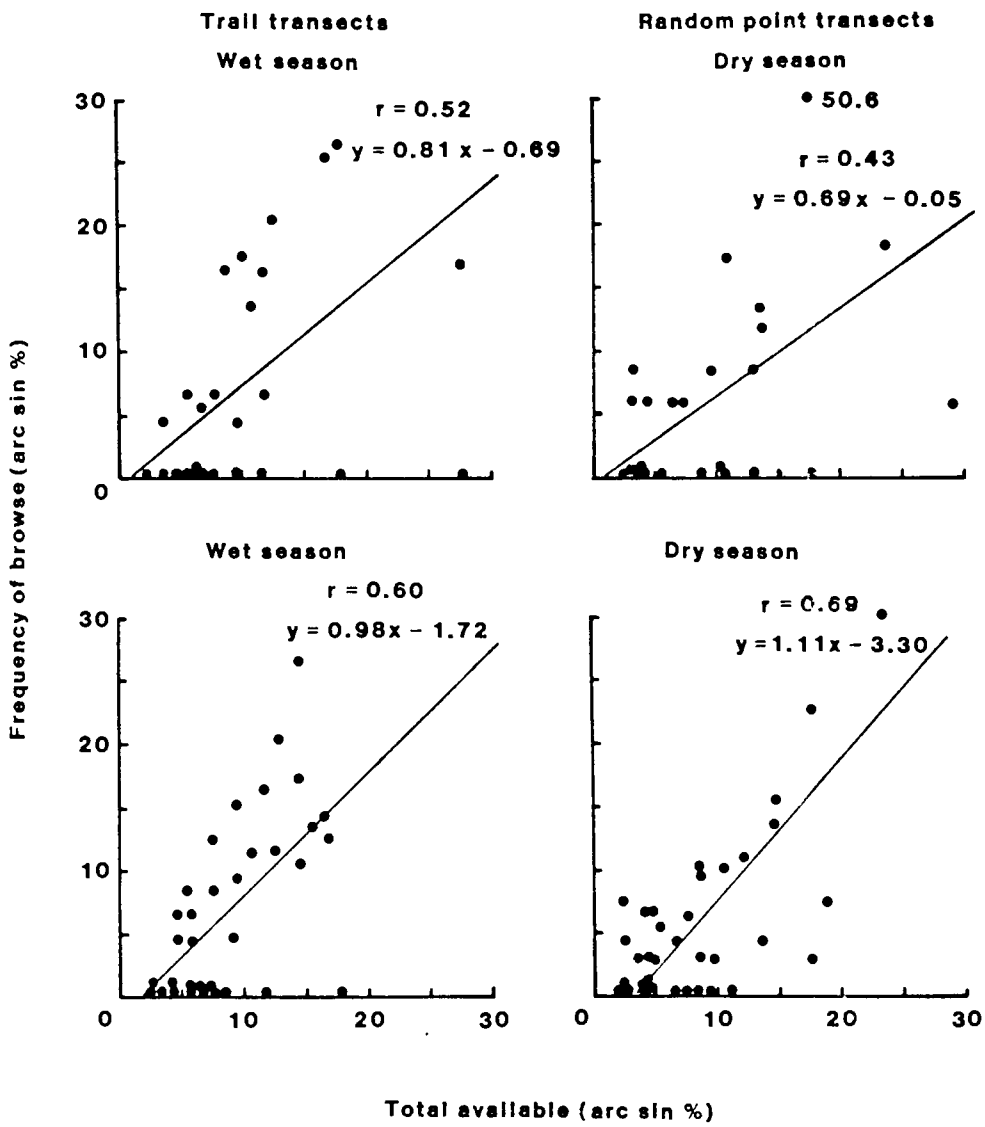


Figure 5.1.6 The relationship between browsed plants and the total number of plants examined for browse on both random point transects and antelope trail transects in wet and dry seasons. The frequency of browse on each plant species was calculated as a percentage of the total browse encountered, as was the frequency of occurrence of the given species. All percentages were arc sin transformed.

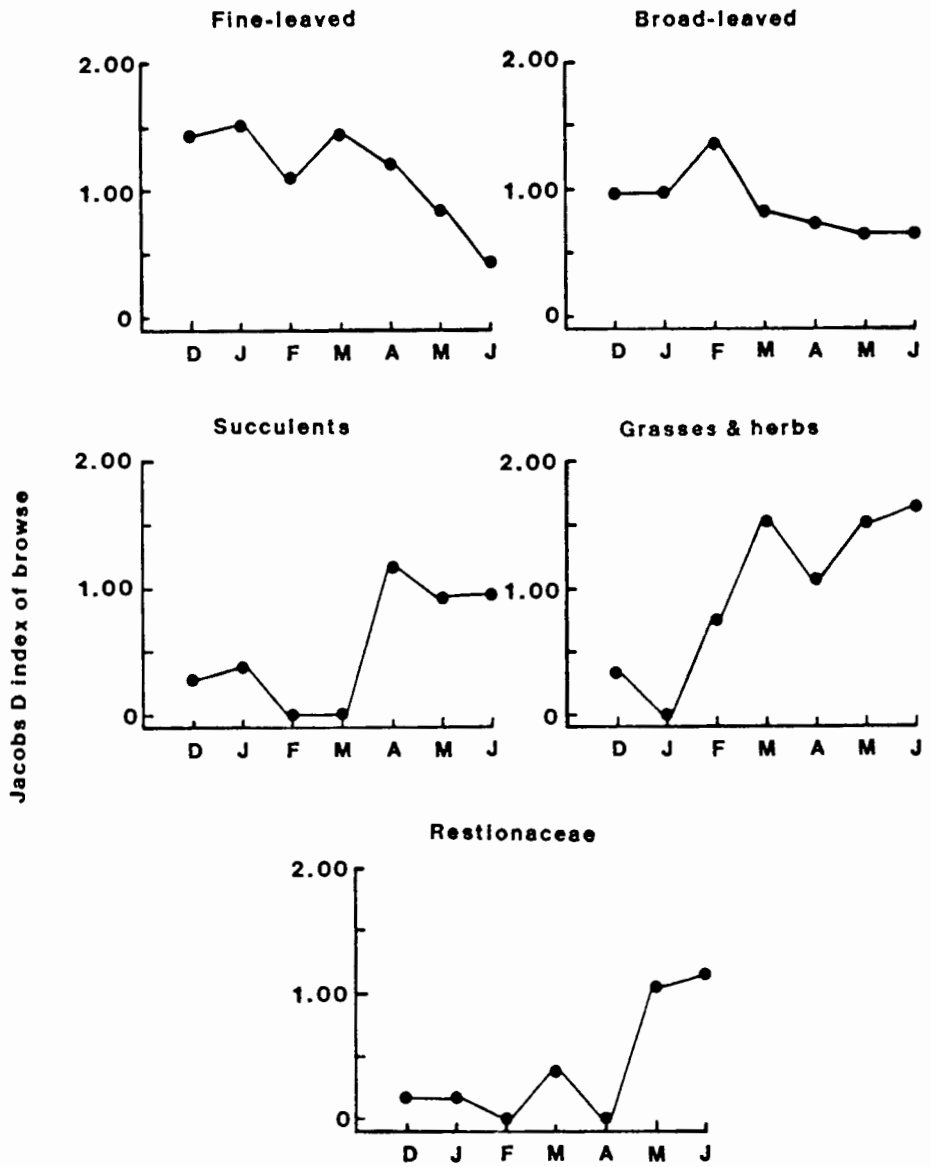


Figure 5.1.7 New browse on perennial plant species in five categories of life form, during a seven-month period from dry midsummer to wet winter in Strandveld vegetation. Jacob's D index values (see text) greater than 1.00 indicate preference by antelope and values less than 1.00 indicate avoidance.

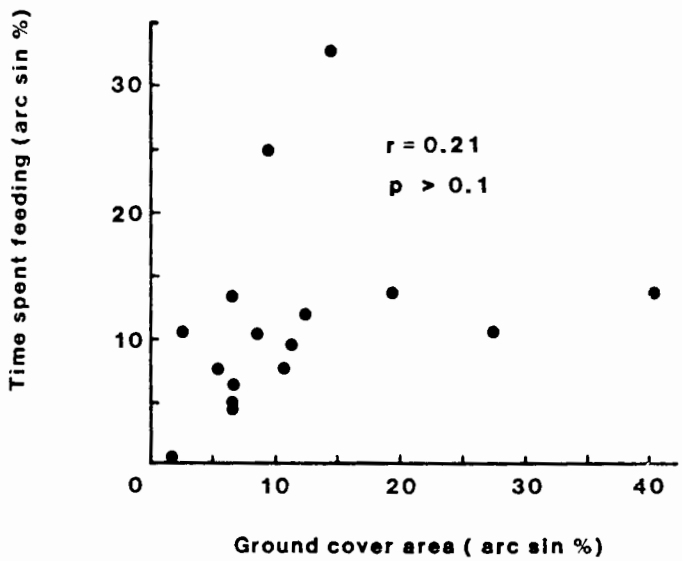
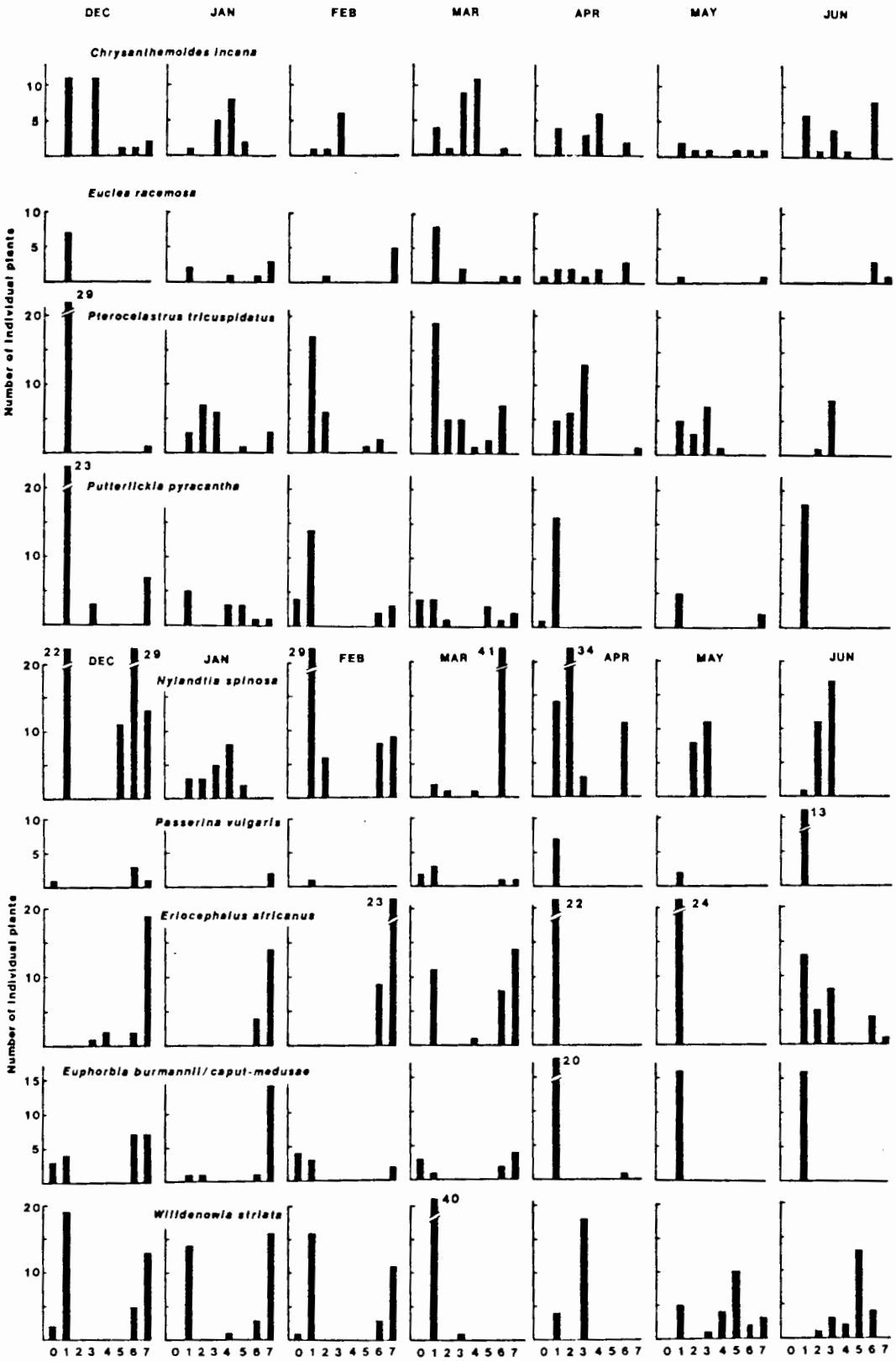


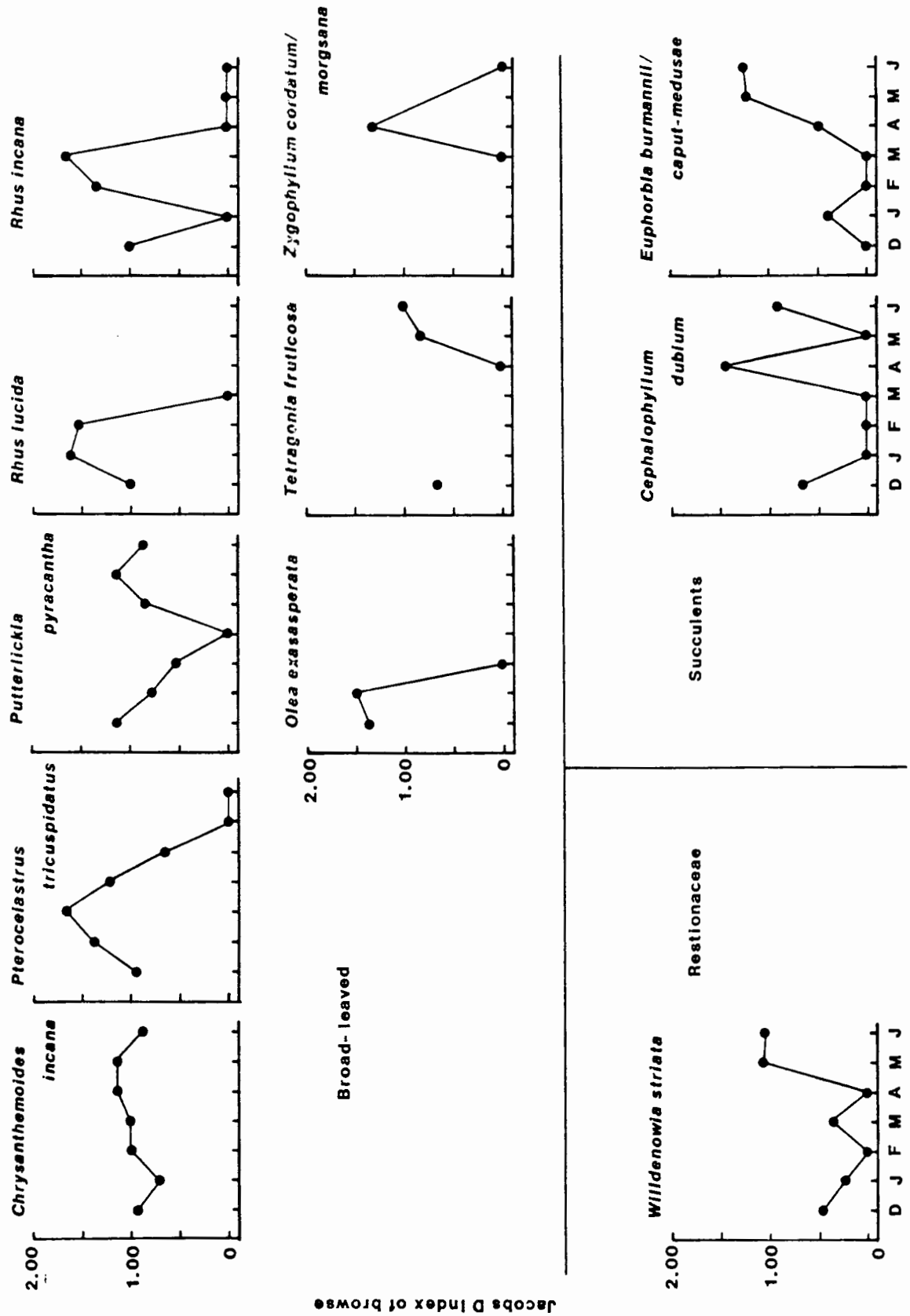
Figure 5.1.8 The relationship between ground cover area of Strandveld vegetation and time spent feeding by captive antelope (Raphicerus campestris steenbok and R. melanotis grysbok) in a field feeding experiment. Both axes are arcsin transformed percentages.

Appendix 5.1.1 Shrubland plants encountered and identified during the antelope browse study.

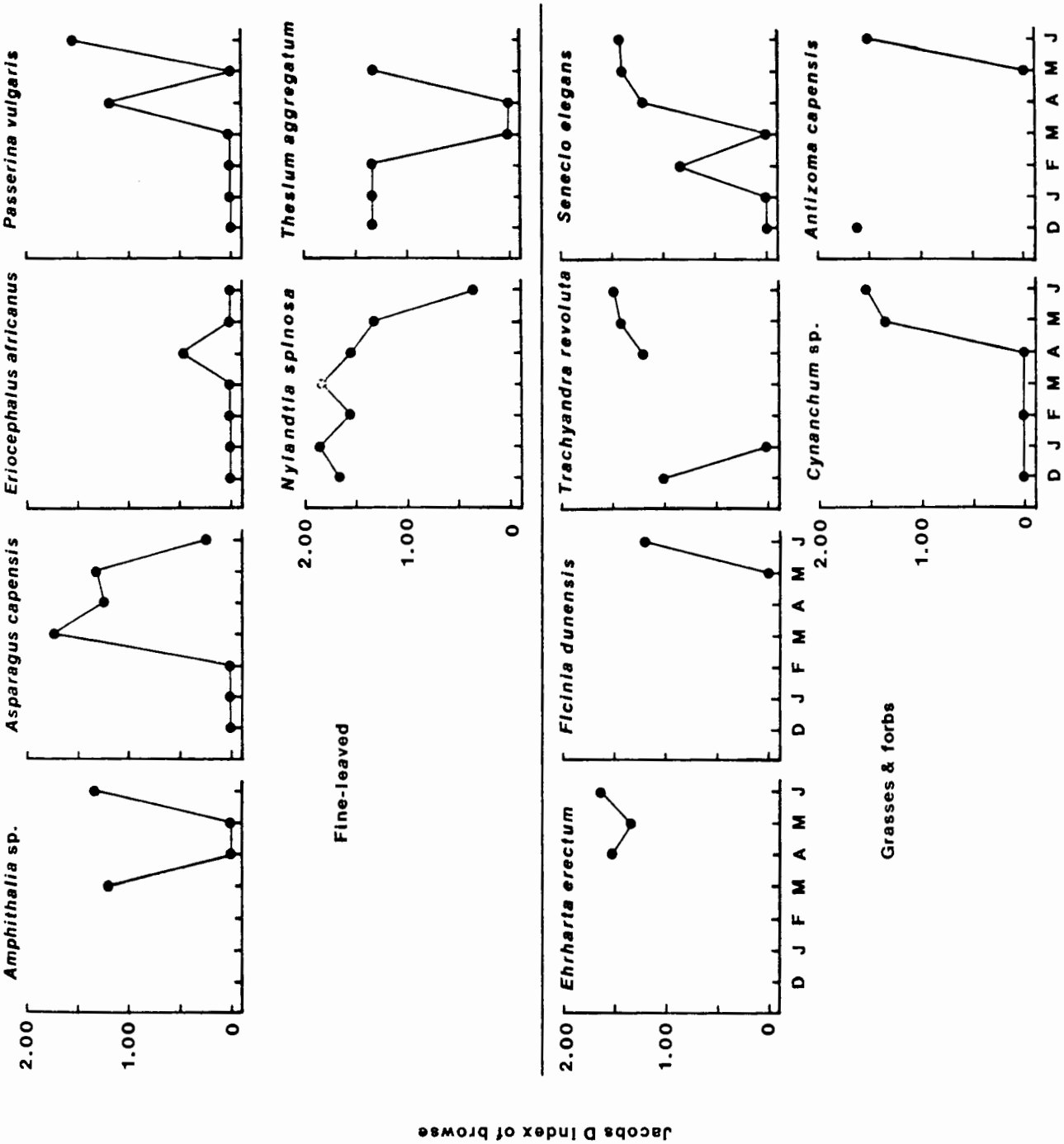
Agathosma imbricata (L.) Willd.
Agathosma serpyllacea (R. & Sch.) Licht.
Amphithalia sp.
Antholyza ringens
Antizoma capensis
Aspalathus hispida
Asparagus aethiopicus L.
Asparagus capensis L.
Carpobrotus edulis (L.) N.E.Br.
Cassytha ciliolata Nees
Centella sp. (glabrata?)
Cephalophyllum dubium
Chrysanthemoides incana (Burm.F.) T. Norl.
Clutia daphnoides Lam.
Colpoon compressum Berg.
Cotyledon orbiculata L.
Crassula rupestris (?) Thunb.
Cynanchum sp.
Ehrharta erecta Lam.
Eriocephalus africanus L.
Euclea racemosa Murr.
Euphorbia burmannii E. Mey. ex Boiss.
Ficinia dunensis Levyns
Haemanthus sp.
Helichrysum crispum (L.) D. Don
Helichrysum cymosum (L.) D. Don
Heliophila sp.
Leucospermum pareli
Leysera gnaphaloides L.
Limonium roseum (Sm.) O. Kze.
Microlomma sagittatum (L.) R. Br.
Muraltia dumosa (Poir.) DC.
Myrsine africana L.
Nylandtia spinosa
Olea exasperata Jacq.
Passerina vulgaris (Meisn.) Thoday
Phylica stipularis L.
Pterocelastrus tricuspidatus (Lam.) Sond
Putterlickia pyracantha (L.) Endl.
Restio eleocharis Nees
Rhus glauca Thunb.
Rhus incana Ait
Rhus lucida L.
Ruschia caroli (L. Bol.) Schwant.
Salvia aurea L.
Salvia nivea
Senecio elegans L.
Stachys aethiopica L.
Struthiola parviflora Bartl.
Tetragonia fruticosa L.
Thesium aggregatum A.W.Hill
Trachyandra revoluta (L.) Kunth
Willdenowia striata Thunb.
Zygophyllum cordatum
Zygophyllum morgsana L.



Appendix 5.1.2 Monthly phenophase diagrams for nine species of Strandveld plants. Phenophase categories: 0 = pregrowth, 1 = new growth, 2 = flower buds present, 3 = flowering, 4 = developing fruit, 5 = ripe fruit, 6 = post-fruiting, 7 = senescent.



Appendix 5.1.3 Monthly changes in amount of browse (Jacob's D indices) on 23 Strandveld plant species grouped as a) broad-leaved species, b) fine-leaved species, c) grasses and forbs, d) succulents and e) Restionaceae.


Amphithalia sp.
Asparagus capensis
Eriocephalus africanus
Passerina vulgaris
Nylandtia spinosa
Theslum aggregatum
Fine-leaved
Ehrharta erectum
Ficinia dunensis
Trachyandra revoluta
Senecio elegans
Cynanchum sp.
Antizoma capensis
Grasses & forbs
2.00
1.00
0
D J F M A M J
Jacobs D Index of browse

5.2 TANNIN POLYPHENOLS IN STRANDVELD PLANTS

5.2 INTRODUCTION

Tannin polyphenols occur in a variety of tissues of many plant species (Bate-Smith 1954, 1957, Swain 1979) particularly in those which become woody during growth (Rhoades & Cates 1976). Generally they are non-toxic feeding deterrents which are effective against a wide range of organisms from insects to mammals. Among vertebrates, they deter feeding in tortoises and lizards (Swain 1979) and influence the food choice of mountain gorillas (Bate-Smith 1972b), chimpanzees (Wrangham & Waterman 1983), howler monkeys (Glander 1978, Milton 1979), vervet monkeys (Wrangham & Waterman 1981) and colobus monkeys (McKey et al. 1981, Oates et al. 1977), snowshoe hares (Bryant 1981), Alaskan ptarmigan, grouse, moose and beavers (Bryant & Kuropat 1980) and Canadian geese (Buchsbaum et al. 1984).

Much of this vertebrate research has been conducted in tropical ecosystems (e.g. McKey et al. 1978, 1981, Milton 1979, Gartlan et al. 1980, Oates et al. 1980, Wrangham & Waterman 1981, McNaughton et al. 1983, McNaughton & Tarrants 1983) and some in subarctic ecosystems (Bryant & Kuropat 1980 and references therein, Bryant 1981, Kuropat & Bryant 1983). Both these ecosystem types are characteristically "stressed" environments with low available soil nutrients and the plants growing in them have responded with particular ecophysiological 'solutions' (Chapin 1980a, 1980b, Bryant et al. 1983). There is increasing evidence that plants in stressed environments produce elevated levels of a variety of classes of secondary metabolites (Rhoades 1979, 1983, Chew & Rodman 1979 and refs.therein, Gershenzon 1984). Beginning with the pioneering work of Ehrlich and Raven (1964), Feeny (1968, 1970) and Feeny and Bostock (1968), theory appears largely to have been derived from plant - insect research

in temperate ecosystems (e.g.Feeny 1976, Rhoades & Cates 1976, Slansky & Feeny 1977, Chew & Rodman 1979, Rhoades 1979, Haukioja 1979, 1980, Rausher 1981, Tempel 1981, Bernays et al. 1981, Bowers 1983, Schultz & Baldwin 1982, Berenbaum 1983, Denno & McClure 1983, Miller & Feeny 1983, McClure & Hare 1984). Some studies have been conducted on tropical plants and insects (e.g. Rehr et al. 1973, Coley 1983)

Very little has been published on vertebrate-plant interactions in mediterranean ecosystems (e.g. Atsatt & Ingram 1983) which like tropical ecosystems, also have characteristically low soil nutrients. In contrast, a fairly large number of studies on insects in mediterranean ecosystems have been published (Mooney et al. 1980, Lincoln et al. 1982, Lincoln & Mooney 1984, Fox & Macauley 1977, Macauley & Fox 1980, Morrow & Fox 1980, Volney et al. 1983).

In order to examine possible chemical determinants of antelope plant preference, plants were collected for chemical analysis of tannin polyphenols and nutritional quality at the same time as antelope selectivity was studied. In this paper I report the individual tannin polyphenol levels of 15 shrubland species, 12 of which were collected seasonally. Sets of values for individual tests are compared with each other. These figures are compared with figures reported in the literature for a number of other southern and east African plants. Some methodological problems are reviewed in the discussion.

5.2 MATERIALS AND METHODS

Collection and extraction of plant material

Approximately 300g of leaf and soft twig material was clipped from a minimum of five individual plants from twelve browsed and unbrowsed plant species. Plant species identifications were checked using Adamson and Salter (1950). In addition, plant ecologists and taxonomists more familiar with strandveld vegetation were regularly consulted about all identifications and updated nomenclature. Collections were made during October 1979, January, April and July 1980. Plant material which had noticeable insect or mechanical damage was not collected. Samples were immediately placed in an insulated box in shade and refrigerated without freezing within a couple of hours. At the earliest opportunity (within 36 hours), the leaves from each species sample were separated from the woody twig and petiole/midrib material and thoroughly mixed. A 3 g sub-sample of this leaf material was then mechanically macerated in pure methanol in a conical flask using a high speed electric homogeniser (Ultra-turrax®). The homogenate was made up to about 45 ml with additional methanol and a two-tier extraction process was then performed (Swain & Hillis 1959).

Extraction involved four changes of 100% methanol and a further three with aqueous (50%) methanol at boiling point (about 65°C for 100% methanol and 75°C for 50% methanol). The two types of extract were decanted separately and retained in the water bath where their volumes were adjusted to 25 ml by evaporation or addition of methanol or aqueous methanol. Each extract was filtered through Whatman's No. 1 cellulose filter paper into volumetric flasks, their volumes were accurately made up to 25 ml and

then transferred into glass bottles with tight-sealing lids for refrigerated storage and later analyses. The remaining fresh leaf material was weighed and oven-dried at 60° C to constant mass and then reweighed in order to determine moisture content. The dry material was then ground in a Wiley Mill to pass through 20 mesh. The ground samples were analysed for nutritional components at a later date and these results are reported in Chapter 5.3.

Analyses

Colorimetric tests were used to measure the concentration of three classes of compounds: total polyphenols, proanthocyanidins and flavanols. The latter two are condensed tannins. Absorbances were determined in a Beckman Spectrophotometer 25. Optimum wavelengths for these readings were determined from absorbance versus wavelength curves. These were generated using the most concentrated standard as a sample (see standards below). A vacuum operated flow-through microcell on the spectrophotometer permitted rapid processing of a large number of samples. Each extract was tested in duplicate or triplicate for each of the three classes of compounds. The results of the pure methanol and aqueous methanol extracts for each species were added together to give a total concentration each test.

Total polyphenols: all the pure methanol and aqueous methanol extracts were tested using the "Improved method" of Singleton and Rossi (1966). The more commonly used method with Folin-Denis reagent(Swain & Hillis 1959, Goldstein & Swain 1963) has some drawbacks which are absent from the Folin-Ciocalteu reaction (Singleton & Rossi 1965). Folin-Ciocalteu test values from a comparison of the two reagents on a

subsample of plant extracts in this study were approximately 10% higher than those from Folin-Denis, but this difference was not significant (Paired t-test, D = -10.7, t = -1.65, df = 21, $p > 0.1$). A standard solution was prepared by dissolving 100 mg tannic acid (Sigma corp.) in 1000 ml distilled water. A standard curve was constructed using various dilutions from full strength to 1 : 10, and this curve was linear. The absorbances were read at 750 nm after a 2 hour colour development period. All results were calculated and reported as tannic acid equivalents (TAE).

Proanthocyanidins : Essentially the same method and reagents as outlined by Swain and Hillis (1959) were used for these compounds. Reaction was carried out in 25 ml wide-necked bottles with screw-caps and rubber gaskets as recommended by Goldstein and Swain (1963). A suitable condensed tannin to use as standard material was unavailable in South Africa when these analyses were being done. Therefore a standard curve generated from quebracho tannin ("Bark Tan" ®, Van Dyke Supply Company, Woonsocket, SD, USA) at a later date in an identical proanthocyanidin determination (Chapter 7.1) was used to calculate concentration of proanthocyanidin from the absorbance values in this study. The same volumes of extract were reacted with the same volumes of reagent under the same conditions using almost identical apparatus. All results were expressed as quebracho tannin equivalents (QTE).

Flavanols: The concentration of flavanols in the extracts was determined using the method of Burns (1971). This is a simpler version of that outlined by Swain and Hillis (1959). Catechin (±) was used as a standard by dissolving 100 mg in 50 ml of pure methanol. Serial dilutions were used to construct a standard curve, which was linear. All test sample determinations were read at a wavelength of 498 nm after 25 min colour

development at 25 ° C. This combination was found to be optimal from experimenting with different combinations of temperature and reaction time (J.P.Glyphis unpublished data). Flavanol concentrations were expressed as catechin equivalents (CE).

Haemanalysis for relative atringency: In this test for relative atringency (Bate-Smith 1973, 1977), fresh diluted animal blood was used as the reagent. Fresh blood (20 ml) was drawn from the cannulated jugular of an adult sheep into a heparinised tube. Within 20 minutes, the blood was diluted 1 : 50 with cold distilled water. It kept refrigerated at 4^{o} C for about 36 hours before the formation of any suspensions. Aliquots (2 ml) of the dilute blood were mixed with 2 ml of appropriately diluted plant extract. This was centrifuged at 3000 rpm for 10 minutes and the absorption of the supernatant was measured at a wavelength of 578 nm. Tannic acid (Sigma corp.) was again used as a standard at a concentration of 1 mg per ml. A standard curve was constructed using dilutions from 0.3 mg to 0.8 mg per ml. This narrow range was necessary to ensure that the stardard curve was linear and the test results were therefore more nearly stoichiometric. All astringency values were calculated and expressed as tannic acid equivalents (TAE).

Tannin polyphenols in other southern African plant species

A single extensive source, the medicinal review of Watt and Breyer-Brandwijk (1962), was systematically searched to compile a list of tannin levels in a much wider sample of southern and east African plants. Qualitative and/or quantitative levels of tannins were obtained for 248 species in 166 genera and 66 families in the following categories: leaves, twigs, roots, bark, reproductive organs and whole plant. The reference for each

entry was recorded using Watt and Breyer-Brandwijk's alpha-numeric system. A list was complied of the relative abundance by species of different common secondary metabolites found in some of the approximately 4415 plant species mentioned in Watt and Breyer-Brandwijk (ibid.).

Statistical analyses

Multiple linear regression analysis (Sokal & Rohlf 1981, Draper & Smith 1981) was used to examine the correlation between the results of the various polyphenol tests. A simple sorting program was used to reduce the plant data from Watt and Breyer-Brandwijk (1962).

5.2 RESULTS

Comparison of polyphenol assays

Folin-Ciocalteu (total polyphenols, Tannic acid equivalents (TAE)) and haemanalysis (astringency TAE) on the 50% (aqueous) methanol extracts produced no significant correlations with any of the other tests on either of the extracts (Table 5.2.1A). The results of 100 % methanol extracts tested with Folin-Ciocalteu and haemanalysis were significantly correlated with results of both proanthocyanidin and flavanol tests on both extracts. The correlation matrix (Table 5.2.1B) indicates that the highest correlations occur between different test methods on the 100 % methanol extracts. These correlation

coefficients vary between 0.68 and 0.79. In contrast there were no significant correlations between the different test methods on the 50 % methanol extracts except for comparison between both types of condensed tannins i.e. proanthocyanidins as quebracho tannin equivalents (QTE) and flavanols as catechin equivalents (CE)), which was highly significant. The significant correlations obtained when comparing each assay result of the two extracts added together are not unexpected since the 100% methanol extract appears to contain from two to five times more polyphenol than the 50% methanol extracts in a majority of the plants analysed.

Seasonal changes in polyphenol levels

Polyphenol concentrations for individual plant species in each of four seasons are tabulated in Appendix 5.2.1. There were significant increases in levels of total polyphenols (Folin-Ciocalteu TAE), flavanols (CE), proanthocyandins (QTE) and astringency (TAE) from winter through to autumn (Table 5.2.2 & 3). Total polyphenols increased two-fold while proanthocyanidins and flavanols increased three-fold. Changes in astringency were not synchronised with changes in the three polyphenol measures: astringency was lowest on average in spring and highest in autumn. Nevertheless, it showed closest approximation to total polyphenols in all seasons except during winter when it was higher. Proanthocyanidin concentrations appeared to be particularly high, in some cases exceeding 100% dw. This is probably ascribable to impurities in the quebracho tannin standard, which may not have been removed in the filtration and recrystallisation purification process.

Differences between new and old leaf material

Comparisons between new and old leaves were possible in eight species and in three of these there was a replicate in a different season (Table 5.2.4). Slightly more than half of the total of 44 ratios calculated for the four measures of tannin polyphenols indicated that new leaves had higher levels than old leaves, while the remainder indicated the reverse. These results, therefore, did not indicate any definite pattern.

Tannin polyphenols in other southern African plants

Comparison of different plant parts (Table 5.2.5) showed that bark contains the highest and leaves the lowest levels of tannins. The concentration of tannin polyphenols (Folin-Ciocalteu TAE) in leaves from the 75 plant samples collected in all seasons ($x = 7.55 \pm 5.17$) in this study was not significantly different from the levels reported in Watt and Breyer-Brandwijk (1962) (Table 5.2.5). In comparing the leaf levels with levels in the rest of the plant, leaves differed significantly in tannin content from bark only (Table 5.2.5).

Occurrence of tannin polyphenols compared with some other secondary defence metabolites in southern African plants

Tannin polyphenols were found in more plant species than any of the four other major classes of compounds (hydrocyanic acid, fixed oils, volatile oils and alkaloids) (Table

5.2.6). This highlights their importance as defense compounds in plants yet much more is known about these other compounds than is known about tannins. Published research on tannins has mostly dealt with tannins as a functional group and relatively little is known about the ecological importance of specific polyphenolic compounds.

5.2 DISCUSSION

I propose 1) to briefly review the historical development of methods for analysing the complexing of polyphenols with proteins, and 2) to discuss the link between low soil nutrient levels and the biosynthesis of polyphenolics.

Polyphenol tannins are very widespread in plants (Bate-Smith 1954, 1969, Bate-Smith and Lerner 1954, Swain 1977, 1979). Based on the small number of structural analyses that have been done, they appear to display great structural diversity (Haslam 1966, 1979, Zucker 1983 and references therein). Their importance as a chemical defence against plant consumers is largely undisputed (Swain 1979, Zucker 1983). According to White (1957), Freudenberg(1920) divided the tannins into hydrolysable and condensed tannins. Hydrolysable tannins have been further divided into three or perhaps four classes : gallotannins (gallic acid + glucose), ellagitannins (ellagic acid + glucose), tara-gallotannins (gallic acid with quinic acid as the core) and possibly the fourth category caffetannins (caffeic acid with a quinic acid core) (Haslam 1966). Condensed tannins are a much more heterogenous group, generally of larger molecular size than hydrolysable tannins. Polyphenol tannins can form strong complexes with proteins, cellulose and starch. Protein binding is due to hydrogen bonding between the peptides on proteins and the phenolic hydroxyls of the tannins (Haslam 1966). The number and arrangement of phenolic groups around the glucose core affects the protein binding capacity of hydrolysable tannins (Haslam 1974). On a per weight basis hydrolysable tannins are usually more astringent than condensed tannins (Bate-Smith 1973). However, the ester bonds of hydrolysable tannins are much more easily cleaved than the carbon - carbon bonds of condensed tannins and this makes condensed tannins less easily degradable (Swain 1979). Although Bate-Smith (1973) suggested molecular

weights of 500 - 3000 as the limits within which tanning will occur, this range may be exceeded at both the upper and lower ends of the scale for nutritional purposes : molecular size can be larger since tannin molecules do not have to penetrate a collagen matrix as in tanning leather, while condensed tannin monomers which are much smaller than 500 daltons might undergo acid condensation during digestion. However, some upper size limit still exists: if the condensed tannin molecules are larger than a certain range, they may not be astringent under most conditions (Zucker 1983).

In most published ecological studies, logistics have made it necessary to analyse for representatives of classes of compounds, e.g. proanthocyanidins as a representative of condensed tannins instead of for individual compounds. The chemistry and methodology of polyphenol assays have been reviewed in some detail (Horvath 1981). In this study, the "Improved method" (Singleton and Rossi 1966) using Folin-Ciocalteu reagent proved to be a more satisfactory method of measuring total polyphenols than the more generally used Folin-Denis method, despite a number of possibly interfering chemicals (Horvath 1981). The absence of carbonate precipitate formation with Folin-Ciocalteu and the reaction stability of this reagent make it particularly suitable for the processing of a large number of samples. Caution should be exercised with both Folin reagents when making comparisons of polyphenol levels in different plant genera and/or species (Swain and Hillis 1959). There does not yet seem to be a polyphenol test reagent which meets the specifications of both chemists and ecologists. More recently, analyses have begun to incorporate some measure of functional activity. This, for tannins, usually entails the measurable precipitation of some soluble protein (Feeny 1969, Bate-Smith 1973). Haemanalysis, using haemoglobin protein (Bate-Smith 1977), appears to be a dependable functional test which is also simple to perform.

The use of a two-tier sequential extraction procedure separates lower molecular weight phenolics from the more complex and more tightly bound compounds : Hillis and Swain (1959) showed with paper chromatography that simple low molecular weight phenolics occurred in the absolute methanol phase while proanthocyanidins occurred in the 50 % aqueous methanol. Goldstein and Swain (1963) suggested that the water breaks hydrogen bonds holding these compounds to other cellular structures and they are not necessarily 'insoluble' in absolute methanol. In this study, the use of two-tier extractions made it possible to make some interesting comparisons of different chemical assays on each of the extracts. The levels of astringency measured by the functional test of haemanalysis in the pure methanol extracts correlated significantly with the levels of total polyphenols (Folin-Ciocalteu TAE), condensed tannins and flavanols in the same extracts. However, astringency (TAE) in the aqueous methanol extracts was not significantly correlated with polyphenol concentrations measured in any of the other three tests and was generally lower in the aqueous methanol than in the pure methanol. This result is consistent with those of Bate-Smith (1973). The aqueous methanol extracts had some astringency which varied from 1.3 to 2.3 % tannic acid equivalents (TAE). The high correlation coefficients of condensed tannins and flavanols is to be expected in view of the close biosynthetic pathway relationship of these two types of compounds (Swain 1979). Extractability of tannin polyphenols still remains a central problem in polyphenol chemistry (Swain 1979).

The biosynthesis of polyphenolics has been linked to plant stress in experimental tissue culture and whole plant studies as well as in field experiments (Chew & Rodman 1979, Rhoades 1979, 1983, Gershenzon 1984). In sycamore cells Acer pseudoplatanus , the levels of total polyphenols (Folin-Denis TAE) increased from approximately 1.5 % d.w. on the fourteenth day to 13.0 % d.w. seven days later (Westcott & Henshaw 1976).

When the tannin levels began to increase in the tissue, the nitrogen levels in the culture medium were almost exhausted. At the onset of tannin synthesis the activity of phenylalanine ammonia-lyase (PAL) increased four fold and then declined sharply. The authors interpret their results as indicating an antagonism between tannin synthesis and nitrogen metabolism. In a whole plant study of sunflowers Helianthus annuus, chlorgenic acid, a phenolic acid product of the shikimic acid pathway, increased 10 - fold with nitrogen deficiency, 6 -fold with water stress and 16 - fold with combined water and nitrogen stress (Del Moral 1972). The author suggestsed that these accumulations alleviate the induced environmental stress and that deterrence against herbivores, while important, is a derived function. In a comparison of several plant species growing under mull (higher soil nutrients) and mor conditions (lower nutrient), Davies et al. (1964) found that condensed tannins (proanthocyanidins) and total tannins as measured by gelatin precipitation from the mor sites were higher. They proposed that the occurrence of tree species will be determined to some extent by the ability of a given species to synthsize polyphenols coupled with differing tolerance of soil nitrogen conditions. Lotus pedunculatus, a grassland cultivar used in New Zealand, contained 8 - 11 % d.w. condensed tannins when grown in acid, low fertility soils and 2 - 3 % d.w. condensed tannins in high fertility soils (Barry and Forss 1983). Fertiliser application to the acid soils reduced condensed tannins to 4 - 5 % d.w. Finally, in a comparison of the polyphenolic content of leaves from two African rain forests, McKey et al.(1978) found that those trees growing in forest on a low nutrient substrate had higher concentrations of polyphenolic compounds. Rhoades (1979) and Gershenzon (1984) have reviewed some other effects of environmental stress on other secondary metabolite levels in plants. These five studies highlight the importance of substrate fertility conditions, a factor which has been neglected in studies of the deterrent role of tannin polyphenols. This should be of special importance in ecosystems with low substrate

nutrients such as tropical forest and all mediterranean-type systems.

Although the mean concentration of total polyphenols is high in the mediterranean shrubland plants in this study, the range is very wide. Therefore, some plants have negligible levels of these compounds while others have extremely high concentrations e.g. Colpoon compressum with 30 % TAE total polyphenol in autumn. This means that in the whole community there is an increased amplitude of variation in the levels of these chemicals because the upper limit has been extended. Many of the species with lower levels appeared to have some other form of defence (personal observation), e.g. thorns and spines in Nylandtia spinosa and Putterlickia pyracantha, latex in Euphorbia spp. and hairy leaves in some of the Rhus spp. and Senecio spp.

The comparison of tannin levels obtained in this study with those obtained from Watt and Breyer-Brandwijk (1962) (Appendix 5.2.2), proved to be interesting. It is remarkable that there was no significant difference between the two means considering that the references in the medicinal covered approximately 5000 species sampled in different seasons and different ecosystems by different workers using a multitude of methods. However an important bias is that much of the earlier research on tannin polyphenols was concerned with finding suitable sources of tannin for the leather industry. Therefore, the values reported were mostly the leaf material from the same plant species in which the bark had already been selected as a likely source of abundant tannin. Not surprisingly, tannins occurred in more plant species in southern and east Africa than four other major classes of defensive compounds, since they have been described elsewhere as the most ubiquitous of the classes of defensive compounds (Levin 1976, Swain 1979). Individual polyphenolics clearly warrant closer attention as ecologically important chemicals than they have received to date (Zucker 1983).

Some of the theory in plant - herbivore interactions predicted that in general older leaves would contain higher levels of quantitative digestibility-reducing defensive compounds such as tannin polyphenols, than younger leaves (Feeny 1976, Rhoades & Cates 1976, McKey 1979). In eight plant species in this study, the differences were equivocal for four different measures of polyphenols. However, an increasing number of studies have shown that new leaves have higher levels of digestibility-reducing compounds than old leaves (e.g. Radwan et al. 1974, Mooney et al. 1981, Lincoln et al. 1982, Coley 1983, Prudhomme 1983).

Table 5.2.1A. Correlation matrix of different tannin polyphenols measures on 62 different plant samples covering approximately 15 different plant species over 4 seasons.

	Poly A	Poly B	Poly Tot	Haem A	Haem B	Haem Tot	Pro A	Pro B	Pro Tot	Flav A	FlavB
Poly B	0.34^{3*}										
Poly Tot	0.97^{3*}	0.54^{3*}									
Haem A	0.72^{3*}	NS	0.70^{3*}								
Haem B	NS	NS	NS	NS							
Haem Tot	0.67^{3*}	NS	0.67^{3*}	0.97^{3*}	0.47^{3*}						
Pro A	0.68^{3*}	NS	0.63^{3*}	0.75^{3*}	NS	0.68^{3*}					
Pro B	0.35^{2*}	NS	0.36^{2*}	0.39^{2*}	NS	0.35^{2*}	0.51^{3*}				
Pro Tot	0.67^{3*}	NS	0.63^{3*}	0.74^{3*}	NS	0.67^{3*}	0.99^{3*}	0.59^{3*}			
Flav A	0.73^{3*}	NS	0.68^{3*}	0.70^{3*}	NS	0.61^{3*}	0.79^{3*}	0.46^{3*}	0.80^{3*}		
Flav B	0.40^{2*}	NS	0.42^{3*}	0.44^{3*}	NS	0.42^{3*}	0.46^{3*}	0.66^{3*}	0.51^{3*}	0.51^{3*}	
Flav Tot	0.73^{3*}	NS	0.68^{3*}	0.71^{3*}	NS	0.61^{3*}	0.80^{3*}	0.48^{3*}	0.80^{3*}	0.99^{3*}	0.56^{3*}

Table 5.2.1B. A subset of data from Table 5.2.1A reduced to simplify comparison of each of the extract types and the totals.

Poly A	Haem A	Pro A	Poly B	Haem B	Pro B	Poly Tot	Haem Tot	Pro Tot
Haem A 0.72^{3*}			Haem B NS			Haem Tot 0.67^{3*}		
Pro A 0.68^{3*}	0.75^{3*}		Pro B NS	NS		Pro Tot 0.63^{3*}	0.67^{3*}	
Flav A 0.73^{3*}	0.70^{3*}	0.79^{3*}	Flav B NS	NS	0.66^{3*}	Flav Tot 0.68^{3*}	0.61^{3*}	0.80^{3*}

A = 100% methanol extract
B = 50% methanol extract
Tot = A + B
Poly = total polyphenols (Folin-Ciocalteu reagent TAE % d.w.)
Haem = Astringency (haemanalysis TAE % d.w.)
Pro = Proanthocyanins (Butanol-HCl hydrolysis,Quebracho Tannin Equivalents(QTE)% dw)
Flav = Flavanols (Vanillin-HCl reagent, Catechin(±) Equivalents (CE) % d.w.)
NS = not significant
^{3*} = $p < 0.001$
^{2*} = $0.005 > p > 0.001$
* = $0.01 > p > 0.005$

Table 5.2.2. Means and standard deviations for four different polyphenol tests on some shrubland plant species measured in each of four seasons.

	Winter (n=17)		Spring (n=21)		Summer (n=15)		Autumn (n=21)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Poly A	3.4	2.5	4.5	4.3	6.2	3.4	9.9	7.2
Poly B	2.3	1.5	1.4	1.1	1.8	1.3	2.1	1.8
Poly Tot	5.7	3.0	5.9	5.0	8.0	4.0	12.0	7.9
Haem A	5.3	3.6	4.4	3.5	6.9	5.2	9.4	6.7
Haem B	2.3	1.6	1.4	1.9	1.3	1.0	1.6	1.6
Haem Tot	7.6	4.1	5.7	4.3	8.2	5.5	10.9	7.4
Pro A	14.8	17.6	22.3	32.3	54.8	64.8	62.3	61.6
Pro B	6.0	5.7	5.4	7.5	4.8	6.1	7.3	7.6
Pro Tot	20.8	22.1	27.7	38.2	59.8	67.7	69.6	65.7
Flav A	5.9	8.5	7.1	9.9	10.9	12.9	21.0	22.4
Flav B	1.3	1.3	0.6	1.2	0.7	0.8	1.4	1.5
Flav Tot	7.2	9.1	7.2	9.9	11.6	13.1	22.4	23.1

A = 100% methanol extract
B = 50% methanol extract
Tot = A + B
Poly = total polyphenols (Folin-Ciocalteu reagent TAE % d.w.)
Haem = Astringency (haemanalysis TAE % d.w.)
Pro = Proanthocyanins (Butanol-HCl hydrolysis,Quebracho Tannin Equivalents(QTE)% dw)
Flav = Flavanols (Vanillin-HCl reagent, Catechin(±) Equivalents (CE) % d.w.)

Table 5.2.3. Change in polyphenol levels in the sampled plant species (%) during four seasons, compared by analysis of variance.

	F	df	p
Total polyphenols	5.73	3, 70	< 0.005
Total astringency	3.18	3, 70	< 0.050
Total proanthocyanidins	4.00	3, 70	< 0.025
Total flavanols	4.49	3, 70	< 0.005

Table 5.2.4. Ratio of polyphenols, flavanols, proanthocyanidins and astringency in new : old leaves. A ratio greater than 1.0 indicates that a particular polyphenol concentration is higher in the new leaves while a ratio of less than 1.0 indicates the reverse.

Species	Season	Ratio			
		Polyphenols	Flavanols	Proanthocyanidins	Astringency
Chrysanthemoides incana	Winter	0.3	0.5	0.7	1.1
	Spring	1.6	5.0	0.1	1.1
Colpoon compressum	Spring	1.4	1.8	1.8	1.2
Euclea racemosa	Spring	0.7	0.3	0.6	0.7
Olea africana	Spring	1.7	1.9	13.6	1.1
Pterocelastrus tricuspidatus	Spring	0.5	0.2	0.2	0.1
Putterlickia pyracantha	Autumn	1.1	1.1	1.3	0.7
Rhus incana	Spring	1.9	9.0	0.8	2.0
	Autumn	0.8	0.9	0.8	0.7
Rhus lucida	Winter	2.1	0.7	66.4	1.8
	Spring	1.8	0.8	1.3	0.9

Table 5.2.5. Comparison of tannin levels in a) leaves from this study (TS) with levels in leaves from Watt and Breyer-Brandwijk (1962) (WBB) and b) of tannin levels in leaves and other plant parts all from Watt and Breyer-Brandwijk (ibid.).

Parts compared	t	df	p
a) leaves (TS) and leaves (WBB)	0.51	94	> 0.1(NS)
b) leaf and twig	0.16	24	> 0.1(NS)
leaf and bark	3.09	80	< 0.01
leaf and roots	0.15	25	> 0.1(NS)
leaf and reproductives	1.49	29	> 0.1(NS)
leaf and whole plant	0.95	40	> 0.1(NS)

Table 5.2.6. Percentages of 4415 plant species mentioned in Watt and Breyer-Brandwijk (1962) which contain probable deterrent chemical compounds.

Deterrent compound	n	%
Tannin polyphenols and other phenols	289	6.6
Hydrocyanic acid	251	5.7
Fixed oils	232	5.3
Volatile oils	197	4.5
Alkaloids	162	3.7

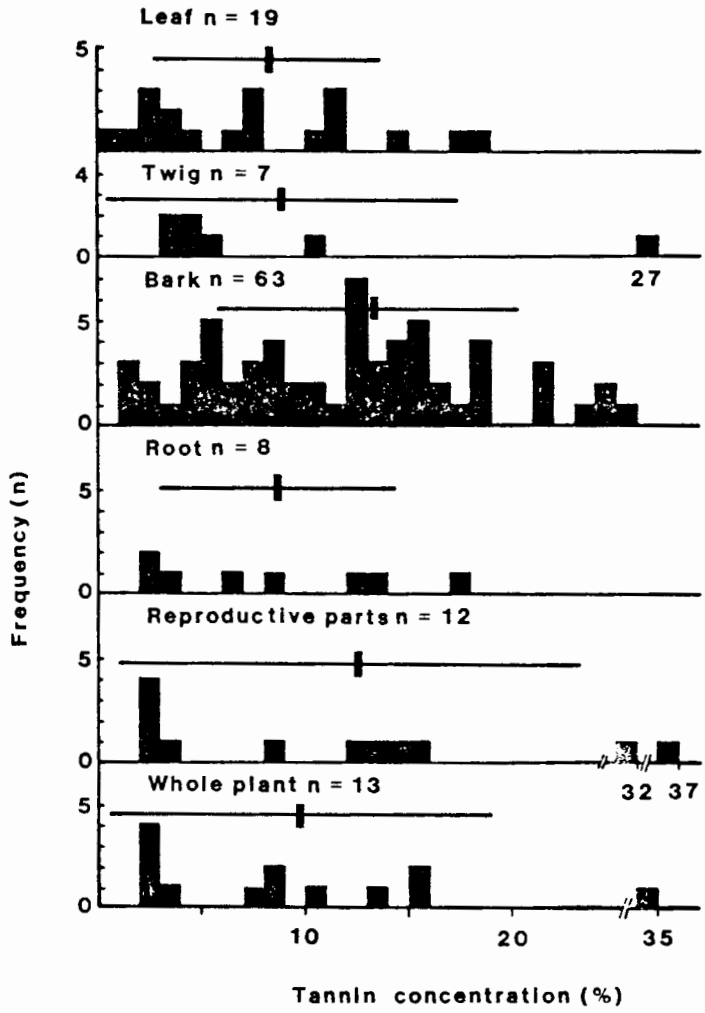


Figure 5.2.1 Frequency distribution of tannin levels in different plant parts in approximately 100 different southern and east African plant species. Means ± one standard deviation are shown with horizontal bars for each category. (Data from Watt & Breyer-Brandwijk 1962).

Appendix 5.2.1. Concentrations of Tannin polyphenols (% d.w.) in leaf material of some South African shrubland plant species measured during four seasons. New (N) and old (O) material were distinguished from each other when both were present in a particular species.

	PolyA	PolyB	Poly Tot	HaemA	HaemB	Haem Tot	Pro A	Pro B	Pro Tot	Flav A	Flav B	Flav T
WINTER												
Colpoon compressum	8.8	4.6	12.8	2.6	2.0	4.6	40.9	13.0	53.9	10.8	1.1	
Chrysanthemoides incana N	0.7	1.3	2.0	4.0	3.0	7.0	1.9	2.9	4.8	0.3	0.6	
O	4.1	2.7	6.8	4.0	2.7	6.7	1.6	5.2	6.8	1.2	0.8	
Eriocephalus africanus	6.9	2.1	9.0	5.9	4.6	10.5	2.7	0.0	2.7	1.0	0.5	
Euclea racemosa	6.3	2.2	8.5	16.1	2.1	18.2	48.2	7.0	55.2	25.9	1.7	
Euphorbia burmannii	2.5	1.2	3.7	4.3	3.6	7.9	0.0	2.0	2.0	1.1	0.0	
Nylandtia spinosa	3.4	0.8	4.2	3.8	0.8	4.6	0.6	0.0	0.6	0.3	1.1	
Passerina vulgaris	0.1	5.1	5.2	6.8	2.0	8.8	10.4	6.2	16.6	1.7	0.8	
Protea repens	3.1	1.4	4.5	2.2	0.0	2.2	19.0	15.3	34.3	4.2	2.2	
Pterocelastrus tricuspidatus	1.2	1.1	2.3	2.0	1.4	3.4	6.2	2.8	9.0	0.9	0.9	
Rhus incana	3.5	1.1	4.6	10.6	2.5	13.1	33.8	8.4	42.2	5.7	2.4	
Rhus lucida N	2.3	4.0	6.3	5.7	5.5	11.2	49.5	16.9	66.4	14.2	5.1	
O	1.2	1.8	3.0	5.2	1.1	6.3	1.0	0.0	1.0	26.2	1.6	
Salvia aurea	2.7	5.7	8.4	5.5	4.3	9.8	2.4	0.0	2.4	1.6	0.2	
Senecio elegans	1.8	2.1	3.9	8.1	0.0	8.1	4.4	11.3	15.7	2.1	0.0	
Thesium aggregatum	7.1	2.3	9.4	3.2	2.0	5.2	23.2	9.7	32.9	1.9	2.7	
Willdenowia striata	1.9	0.5	2.4	1.4	0.9	2.3	6.5	0.8	7.3	1.1	0.0	
SPRING												
C. compressum N	17.8	3.1	20.9	11.4	0.0	11.4	123.6	15.2	138.8	36.0	0.1	
O	12.0	2.6	14.6	9.8	0.0	9.8	63.5	13.0	76.5	18.4	0.7	
R. incana N	1.6	3.6	5.2	1.7	1.9	3.6	1.1	0.0	1.1	0.5	0.0	
O	2.0	1.2	3.2	1.9	1.4	3.3	1.1	0.9	2.0	0.1	0.0	
E. africanus	6.0	1.3	7.3	9.2	2.1	11.3	1.1	0.0	1.1	0.7	0.4	
E. racemosa N	5.3	1.4	6.7	2.8	4.0	6.8	21.1	2.6	23.7	7.0	0.0	
O	7.6	2.4	10.0	8.1	1.8	9.9	34.9	7.4	42.3	25.5	0.6	
E. burmannii	4.4	0.8	5.2	6.1	0.0	6.1	55.2	3.3	58.5	6.9	0.0	
Haemanthus sp.	0.4	0.4	0.8	0.0	0.0	0.0	2.3	0.0	2.3	0.2	0.0	
N. spinosa	1.1	0.1	1.2	1.1	0.0	1.1	0.6	0.0	0.6	0.1	0.1	
Olea exasperata N	7.3	0.5	7.8	3.7	0.0	3.7	0.7	0.7	1.4	2.9	0.2	
O	4.0	0.4	4.4	2.5	0.9	3.4	1.1	0.0	1.1	1.4	0.2	
P. vulgaris	1.7	1.3	3.0	2.8	1.8	4.6	2.7	4.6	7.3	0.5	0.9	
P. tricuspidatus N	1.1	0.8	1.9	0.0	0.0	0.0	8.2	3.1	11.3	2.8	0.0	
O	2.3	1.9	4.2	2.9	0.0	2.9	36.4	11.1	47.5	11.9	1.1	
R. incana N	1.7	0.6	2.3	4.0	0.9	4.9	4.7	2.2	6.9	1.8	0.0	
O	0.7	0.6	1.3	1.3	1.1	2.4	0.7	1.9	2.6	0.1	0.2	
R. lucida N	5.5	2.9	8.4	6.0	2.6	8.6	59.7	25.7	85.4	17.8	4.6	
O	4.1	0.8	4.9	6.9	2.1	9.0	47.3	20.8	68.1	12.5	3.7	
S. aurea	7.7	2.7	10.4	8.7	7.9	15.6	1.4	0.0	1.4	0.9	0.5	
W. striata	1.0	0.1	1.1	1.1	0.0	1.1	1.2	0.4	1.6	0.2	0.0	

Cont.

Appendix 5.2.1 (cont.)

SUMMER											
C. compressum	13.0	4.3	17.3	20.5	2.2	22.7	192.9	14.0	206.9	10.2	1.8
C. incana	3.7	3.1	6.8	2.8	2.1	4.9	1.6	0.0	1.6	1.1	0.0
E. africanus	4.5	2.0	6.5	2.9	2.3	5.2	1.1	0.0	1.1	1.1	0.2
E. racemosa	3.8	1.0	4.8	12.2	0.0	12.2	23.5	3.8	27.3	11.1	0.5
E. burmannii	9.7	0.2	9.9	6.5	0.0	6.5	58.6	3.8	64.4	11.1	0.0
N. spinosa	6.0	2.4	8.4	3.9	0.0	3.9	0.7	0.0	0.7	0.3	0.3
O. exasperata	2.9	0.6	3.5	3.1	1.0	4.1	1.2	0.0	1.2	2.4	0.0
P. vulgaris	1.9	1.6	3.5	3.3	2.6	5.9	22.8	19.9	42.7	4.2	1.6
P. tricuspidatus	8.2	0.4	8.6	2.8	0.0	2.8	133.8	1.5	135.3	21.4	0.0
Putterlickia pyracantha N	9.4	2.3	11.7	10.8	2.0	12.8	139.2	12.4	151.6	19.6	2.1
R. incana	7.3	1.5	8.8	7.0	1.4	8.4	66.8	6.7	73.5	16.3	1.5
R. lucida	11.4	2.0	11.4	11.3	1.7	13.0	135.3	5.8	141.1	49.0	1.2
S. aurea	4.5	4.5	9.0	10.1	2.4	12.5	1.9	0.0	1.9	0.7	0.1
T. aggregatum	3.1	1.1	4.2	1.8	1.3	3.1	6.4	0.0	6.4	0.5	1.5
W. striata	3.4	0.4	3.8	4.2	0.0	4.2	36.7	4.4	41.1	14.4	0.3
AUTUMN											
Aspalathus hispida	13.6	7.3	21.0	5.7	0.0	5.7	2.2	0.6	2.8	1.8	0.1
Asparagus capensis	2.0	0.5	2.5	2.8	0.0	2.8	1.1	0.0	1.1	1.7	0.1
C. compressum	30.2	1.8	33.0	24.2	0.0	24.2	200.6	15.8	216.4	66.1	1.2
Cotyledon orbiculare	3.8	0.5	4.3	17.3	3.1	20.4	63.2	3.7	66.9	11.0	0.4
C. incana	3.8	1.6	5.4	2.5	1.7	4.2	1.3	1.0	2.3	1.3	0.7
E. africanus	8.1	2.3	10.4	11.2	2.6	13.8	1.8	0.0	1.8	1.4	1.5
E. racemosa	12.7	2.9	15.6	13.1	0.0	13.1	70.2	10.3	80.5	33.6	1.8
E. burmannii	8.1	0.2	8.3	14.0	6.7	20.7	128.6	3.8	132.4	14.6	0.1
N. spinosa	1.8	0.2	2.0	1.3	0.8	2.1	0.9	0.0	0.9	0.4	0.1
P. vulgaris	5.0	4.8	9.8	3.1	2.0	5.1	36.7	25.6	62.3	8.4	2.5
P. stipularis	9.7	2.3	12.0	8.5	1.9	10.4	69.1	21.6	90.7	25.5	4.0
P. tricuspidatus	6.3	2.0	8.3	9.0	2.5	11.5	83.5	11.6	95.1	18.4	1.0
P. pyracantha N	18.8	1.1	19.9	2.9	0.0	2.9	93.3	4.4	97.7	55.0	0.8
O	21.6	1.7	23.3	11.7	1.6	13.3	80.0	8.3	88.3	57.3	1.6
R. incana N	9.1	2.9	12.0	11.4	1.6	13.0	113.9	12.5	126.4	33.8	3.3
O	13.2	2.3	15.5	17.7	2.0	19.7	153.3	6.4	159.7	41.0	2.0
R. lucida	13.6	3.2	16.8	20.4	2.1	22.5	161.2	14.8	176.0	56.5	5.5
S. aurea	9.9	4.0	13.9	10.7	2.7	13.4	2.0	0.0	2.0	1.5	0.6
T. aggregatum	11.3	2.4	13.7	2.2	1.6	3.8	20.5	10.1	30.6	2.2	2.4
W. striata	3.4	0.4	3.8	2.0	0.0	2.0	22.2	2.4	24.6	8.1	0.3

A = 100% methanol extract
B = 50% methanol extract
Tot = A + B
Poly = total polyphenols (Folin-Ciocalteu reagent TAE % d.w.)
Haem = Astringency (haemanalysis TAE % d.w.)
Pro = Proanthocyanins (Butanol-HCl hydrolysis,Quebracho Tannin Equivalents(QTE)% dw)
Flav = Flavanols (Vanillin-HCl reagent, Catechin(±) Equivalents (CE) % d.w.)

Appendix 5.2.2 Tannin polyphenols in approximately 240 species in 160 genera and 65 families of plants occurring in southern and east Africa. This data was extracted from Watt and Breyer-Brandwijk (1962) and the Reference number quoted refers to their alphanumeric reference list.

Family and species	Tannin polyphenols Plant part (%)	Other secondary compounds	Ref. No.
Acanthaceae			
Thunbergia capensis	leaf(+)		G19
Anacardiaceae			
Anarcardium occidentale	tree(+)		M194
Heeria argentea	leaf, twig(4.8-4.9)		G19,J76
Harpephyllum caffrum	leaf, twig(11.4)		W77
Mangifera indica	stem,bark,leaf,twig(+)		B251
Rhus cotinus	leaf(18-20),blossom(3-16)		V122
R. incana	bark(19.2)		W77
R. laevigata	bark(18.6),leaf(4.0)		W77
R. lucida	bark,wood		D22
R. tomentosa	leaf(8.0),twig(5.7)		D22,W77
Sclerocarya caffra	bark(10.7-20.5)		W77,B34
Sonodingium argutum	bark		W77
Annonaceae			
Annona chrysophylla	green fruit(+++)		S537
A. reliculata	seed kernel,bark(+)		Q2,Q4,V30,W112
A. squamosa	root(+)		G19
Apocynaceae			
Carissa bispinosa	leaf,fruit(+)		W159
Gonioma camassi	bark(3.8)		J11
Vinca major	leaf(+)		S53
V. minor	leaf(+)		B39,R225,P1
Bombacaceae			
Adansonia digitata	vascular sap(+)		A287,A288
Burseraceae			
Cavarium schweinfurtlii	bark(0.7)		I25
Cactaceae			
Opuntia ficus-indica	leaf(+)		H31
Canellaceae			
Warburgia stuhlmanii	bark(+)		W37
Capparidaceae			
Crataeva religiosa	bark(+)		W37
Casuarinaceae			
Casuarina cunninghamiana	bark(6.7-11.3)		W77
Celastraceae			
Pterocelastrus rostratus	bark(2.1),leaf(+)		S87,J11
P. tricuspidatus	bark(5.1-6.1)		J11

cont.

Cassine uroceum	bark(16.4)		W77
Catha edulis	leaf(+)		P309
Gymnosporia buxifolia	bark(+)		G19
G. senegalensis	bark		G19
Pseudocassine transvaalensis	bark (13.3)		T2
Combretaceae			
Combretum zeyheri	fruit(17.8),seed(12.9)		S325
Terminalia catappa	fruit(6-20),leaf,flower(+)		W37,D22,W197
Compositae			
Centaurea picris	whole plant(3)		G390
C. solstitialis	whole plant(1.8),flower(3.3)		M494
Cnicus benedictus	whole plant(+)		G14,T6
Helichrysum nudifolium	whole plant(+)		W324
Matricaria chamomila	+		M26
Oldenburgia arbuscula	+		G54
Taraxacum officinale	whole plant(2.8)		Z3
Convolvulaceae			
Cuscuta epithymum	+		B171,M37
Ipomoea purpurea	whole plant(+)		P27,P260
Crassulaceae			
Crassula argentea	+		G19
C. lycopodioides	+		G19
Cruciferae			
Capsella bursa-pastoris	+		K14
Cucurbitaceae			
Colocynthis citrullus	seed(+)		F218
Telfairia pedata	seed shell(+++)		T192
Cunoniaceae			
Cunonia capensis	bark(8.4)		J11
Platylophus trifollatus	bark(6.1)		J11
Dichapetalaceae			
Dichapetalum cymosum	leaf(+)	fluoroacetate	S101
Dioscoraceae			
Diascorea bulbifera	tuber(+)		W112,W159
D. continifolia	tuber(+)		W159
D. retusa	tuber(+)		W159
D. elephantipes	tuber(+)		V60,W89
Ebenaceae			
Diospyros mespiliformis	fruit(+++),leaf,root(+)		G19
Euclea fruticosa	whole plant(+)		T78
E. undulata	bark(3.3)		J11
Royena lucida	bark(4.7)		J11
Ericaceae			
Agamia salicifolia	bark,leaf(+++)		D150
Acalypha indica	whole plant(+)		W37
Alevrites fordii	leaf(+)		M1
Croton sylvaticus	bark(2.7)		W77
Fluggea virosa	bark,plant(+)		G19,C201,C221
Hyaenanche globosa	leaf,stem(+)		H190
Jatropha curcas	latex(+++)		J24,U51

Euphorbi

cont.

J. zeyheri	tuber(20.5)		W77
Mercurialis annua	tuber(peeled)(3.4)		F126
Phyllanthus discoideus	bark (9.2-10.4)		W77
Fagaceae			
Quercus sp.	bark(9.3-10.1)		W77
Gentianaceae			
Swertia chirata	whole plant(+)		O2
Geraniaceae			
Geranium canescens	root(+)		G19
Monsonia ovata	whole plants(35)	pyrogallol tannin	M207
Pelargonium pulveratum	root(+)		G19
Sarcocaulon burmanii	whole plant(+)		G19
Guttiferae			
Allenblackia floribunda	seed(+)		W37
Garcinia gerrardia	bark(11.3)		W77
Hypericum perforatum	stem(3.8),leaf(12.4),flower (16.2),seed(12.1),whole plant(8.2-9.3)		C326,N56,N60, H60
Hydnoraceae			
Hydnora africana	whole plant(+)		M11,U9
Iridaceae			
Watsonia spp.	whole plant(+)		W159
Labiatae			
Marrubium vulgare	whole plant(+)		C353,A365
Lauraceae			
Ocotea bullata	bark(3.2-5.8)		C1,S11,W37
Thymus vulgaris	leaf,flower(+)		M411,U43
Cinnamomum cassia	bark(+)		D179,H146,M178
C. zeylanicum	bark(+)		M178
Persea americana	seed,leaf,root(+)		V46,V83,W159
Lecythidaceae			
Barringtonia racemosa	stem,root(++)		M11
Leguminosae			
Acacia albida	bark(2-28),fruit(5-14.3)		G43,S325
A. bussei	bark(17-21)		G43
A. catechu	wood(++)		G43
A. caffra	bark(+)		G19
A. cyclops	bark(6.5-12)		J68,W77
A. cyanophylla	bark(16-19)		W314
A. decurrens	bark(24-42)		W77,W79,L308
A. d. var. dealbata	bark(16-20)		S255,S325,W37
A. elata	bark(20-31)	alkaloid	W145
A. farnesiana	fruit(11-33),seed(12)		T6,W145
A. karroo	bark(19),fruit(+)		P3,W37,W77
A. longifolia	bark(7.6-18.9),leaf(1.9)		W77,G43,W145
A. melanoxylon	bark(11-17.6),leaf(3.4)		W77,W145
A. nigrescens	bark(15)		G43
A. nilotica	pod(22.8-38.9),pod case (19-41.7)leaf in inflorescence(+++) bark(14.7-33.4)		A278,A282,R267, W37

cont.

A. nilotica kraussiana	bark(12-14)	catechol	B430,R261
A. nilotica subalata	deseeded pod(30-45)		A345
	pod(20-26.7)		A273,G43
A. pennata	bark(8.8)		W145
A. podalyriaefolia	bark(12.4)		P22,W37
A. pycnantha	bark(18.3-40),leaf(15.2)		J11,J68,W145
A. retinens	galls(9.5)		W77
A. saligna	bark(19.1-30.3)		W77,J11,J68
A. seyal	bark(20)		A224,G19,W145
A. seyal var. fistula	bark(20)		S325
A. sieberiana	bark(+)		G19
A. suma	heart wood(11)		G43
A. welwitschii	gall(5),seed pod(2.8)		W77
Albizia adiantifolia	pod(3.5)		W77
A. lebbeck	bark(7-11)		W37,D22,R101
A. versicolor	bark(4.8)		W77
Calpurnia subdecaudia	leaf,twig(4.4)		W77
Baikiaea plurijuga	wood(8.6-20)	phlobaphenes	G180
Bauhinia fassoglensis	leaf,fruit(+)		W195
Brachystegia longifolia	bark(7-9.4)		B436
Caesalpinia coriaria	pod(30-80)		D22,G43,W37
C. pulcherrima	wood(3),leaf(7)		G43
	pod(+++),leaf,flower		D22,V31,W37
	fruit(+)		W159
Cassia abbreviata	root,bark(+)		B179,G19,R282
C. aschrek	whole plant(+)		G19
C. auriculata	bark(20-24),twig(4.6)		W147,D22,G43
C. slamea	bark(2-9)		B38
C. didymobotrya	bark(+)	anthraquinones	G19
C. mimosoides	leaf(+)		G19
C. occidentalis	seed(+)		B15
C. petersiana	leaf(+)		G19
C. singueana	bark(+)		D22,G43,W147
Ceratonia siliqua	bark(50),trunk,twig(+)		W37
	fruit,flower(++)		W159
Colophospermum mopane	bark(5.9-8.7),leaf(+++)		W195
Cyclopia genistoides	bark(30.3)		W77
C. subternata	whole plant(+)		C87
Elephantorrhiza burkei	root,bark(+)		G19
E. elephantina	root(5.8-22.3)		A87,A240,J7,M10
	root bark(25-30)		W37,D22
Entada africana	leaf,bark(+)		G19
E. phaseoloides	bark(+)		G19
Gleditschia triacanthos	pod,seed coat(3.1),leaf(+++)		W43,W159,R305
	heart wood(4)		
Mimosa pigra	root(10)	mimosine	D22,W144
Hoffmanseggia melanosticta	stem(25-30)		W37

cont.

Millettia sutherlandi	bark(2.2)		W77
Parkia filicoidea	bark(12-14)		G43,W144
	fruit envelope(+)		W144
P. africana	bark(12-14),pod(++)	parkine(alkaloid)	G43,W144
Piliostigma thonningii	bark,small twig(20)		G43,W140
	root,branches(18)		
Prosopis africana	bark(14-16)		G43
P. chilensis	bark(+), root(6-7)	saponins	G43,D22,W37
Pterolobium exosum	bark(+)		G43
Schotia brachypetala	bark(+)		W50
S. afra	bark(16.3)		W77
Tamarindus indica	seed envelope(32),bark(+)		R137,W144
Liliaceae			
Aloe daryana	leaf(+)		W159
Asparagus officinalis	whole plant(+)	vanillin(glucoside)	D22,T32,W37
Scilla saturata	bulb(++)	glucoside	W159
Urginea burkei	whole plant(+)		J14,W261
Loganiaceae			
Nuxia floribunda	bark(5.7)		J11
Lythraceae			
Sonneratia acida	bark(+)		Q4
Woodfordia floribunda	flower(20.6),leaf(10)		W37
Meliaceae			
Ekebergia capensis	bark(7.2)		J11
Khaya senegalensis	bark(10.2)		G47
Melia azedarach	bark(18.3-26)		J11
Ptaeroxylon obliquum	bark(+)		P213,P286
Trichila roka	bark,leaf(+),bark(6-27)		B261,J58,W77
Moraceae			
Chlorophora excelsa	bark(1.6)		I25
Ficus vogelii	latex(+)		G19,W361
Musaceae			
Musa paradisiaca	fruit pulp(+)		B398
Myristicaceae			
Cephalosphaera usambarensis	resin(+)		B256
Myrsinaceae			
Rapania melanophloeos	twig(4.9),bark(11.6-16)		J11,W77
Myrtaceae			
Psidium guajava	leaf(8.2),bark(10-30)		W37
	root(+++),flower(+)		
P. pomiferum	leaf(+),bark(12-15)		W37
Ochnaceae			
Ochna arborea	bark(8.2)		J11
Olacaceae			
Ximenia americana	bark(16.9),leaf(+)		A289,G19
X. caffra	leaf(+)		G19
Oleaceae			
Ligustrum vulgare	bark(7)		G259,M36,P1
Olea europaea	unripe fruit,leaf(+)	glucoside	D146,D22,E62

cont.

Olinaceae			
Olinia radiata	bark(17.2)	hydrocyanic acid	W77,H89
Papaveraceae			
Phoenix redinata	root(+)		G19
Pedaliaceae			
Sesamum indicum	leaf(+)		G19
Phytolacaceae			
Giesekia pharnaceoides	whole plant(+)		C138
Phytolacca dioca	wood(+)	saponin	D96
P. dedecandra	fruit(+)	saponin	K51
Polygalaceae			
Securidaca longipedunculata	root,bark(+)	saponin	G19
Polygonaceae			
Rumex acetosa	whole plant(+),rhizome (2.8-22.6)		S512
R. cordatus	leaf,calyx(1.4),stalk (0.26),rhizome(10-10.9)		J69,M10,W77
R. lanceolatus	rhizome(6.7)		W77
R. nepalensis	rhizome(4.2)		W77
Portulacaceae			
Portulacaria afra	whole plant(++)		M11
Proteaceae			
Brabejum stellatifolium	bark(13.3)	cyanogenic glucoside	W77
Faurea macnaughtonii	whole plant(14)		S83
Grevillea robusta	resin(+)	hydrocyanic acid	Q2,Q4
Leucodendron argenteum	bark(16)		W37
Leucospermum conocarpo-dendrum	bark(12.6)		W77
Mimetes lyrigera	bark(++++)		M11
Protea caffra	bark(12.4-13.2)		W77
P. grandiflora	bark(13.6)		W77
P. hirta	bark(++++)		S83
P. lepidocarpodendron	bark(12.6)		B99,M11,P2
P. mellifera	bark(3),whole plant(16.4)		W37,W77
Punicaceae			
Punica granatum	fruit rind,bark(++++) leaf(+++)	alkaloids	D2,W159
Rhamnaceae			
Helinus ovata	leaf(+)		G25
Ziziphus mucronata	bark(12.2-15.7)		W77
Rhizophoraceae			
Brugviera gymnorrhiza	whole plant(++),leaf(11.9) bark(12.8-53.1)		W77,D148
Rhizophora mucronata	whole plant(+++) bark(4-48)		W37,W77
Rosaceae			
Agrimonia eupatoria	whole plant(2.6)	glucoside	G165
Eriobotrya japonica	leaf,fruit(++)		V31,W159
Prunus persica	leaf(0.8-2.4)		W37
Pyris communis	leaf(8.1)	hydrocyanic acid	G14,K52,P92
Rubus pinnatiformis	root(+)		G19
R. rigidus	root(+)	cont.	G19

Rubiaceae			
Asperula adorata	fresh herb(++)		W37
Richardia brasiliensis	root(++),plant(+)		W37
Rutaceae			
Citrus aurantium	fruit rind(+)		N28,Q4
Fagara capensis	root,twig(+)		J4
Vepris lanceolata	bark(2)		J11
Santalaceae			
Osyris compressa	leaf(11-26)		W79
Thesium hystrix	root(3.2)		S40
Sapindaceae			
Cardiospermum halicacabum	leaf(+)	cyanogenic glucoside saponin	Q3
Dodonea viscosa	leaf(+)	alkaloid,saponin glucoside	Q3,W261,W3
Solanaceae			
Physalis peruviana	husk(+)	saponins	L219
Solanum auriculatum	heartwood(0.45)	gluco-alkaloid	P329
S. campylacanthum	fruit(2.5)		A373
S. tuberosum	leaf(3.2-3.4)		M432
Sterculiaceae			
Heritieria littoralis	bark(13.9)		W37
Taxaceae			
Podocarpus falcatus	bark(2.8-6.1)		J11-W77
P. latifolius	bark(2.7-3.6)		J11-W77
Thymelaeaceae			
Lasiosiphon meisnerianus	bark(++)		R34
Grewia excelsa	root(+)		G19
G. forbesii	root(+)		G19
Ulmaceae			
Trema guineënsis	wood(+)		G19
Umbelliferae			
Centella coriacea	whole plant(8.9)		W37
Verbenaceae			
Avicennia officinalis	bark(++)		D22,D180
Lantana camara	root(+)		Q4
Priva cordifolia	seed(+)		G19
Violaceae			
Viola tricolor	flower,seed(+)	saponin	W37
Vitaceae			
Cissus adenocaulis	root(+)		G19
C. quadrangularis	root,leaf(+)		G19
Vitis cirrhosa	root(+)		G19
V. hildebrandtii	root(+)		G19
Filices			
Pteridium aquilinum	rhizome(+++)		E13

5.3 THE ROLE OF TANNINS AND NUTRITIONAL COMPONENTS IN BROWSE SELECTION BY SMALL ANTELOPE IN STRANDVELD VEGETATION

5.3 INTRODUCTION

In Chapter 5.1, I presented evidence that small ruminants are very selective feeders in a species-rich and nutrient-poor vegetation. Patterns of selectivity were described both on the basis of plant life form and on individual plant species. Although it is generally accepted that plant palatability moderates patterns of selectivity in herbivores (Harper 1969, Kulman 1971, Rauscher & Feeny 1980) there is considerable debate as to which plant characteristics, or combinations of characteristics, are ultimately responsible (Arnold & Hill 1972, Laycock 1978, Marten 1978, Westoby 1978, Schluter 1981, Bryant & Kuropat 1980). Some work supports nutritional quality while more recently there is increasing evidence that avoidance of plant secondary metabolites is the key factor in palatability(Bryant & Kuropat 1980 and references therein). The array of both nutritional, toxic or deterrent compounds in any given plant tissue is large and many of these will be implicated in the decision of an animal to feed on a given tissue (Westoby 1978, Pulliam 1975, Freeland & Janzen 1974).

Most studies have compared a restricted array of nutritional components when both deterrent and beneficial elements have been included in analysis. However, multiple regression suggests itself as a suitable tool for analysis of these factors because of its capacity to handle large arrays of variables. It has been successfully used, for example, in a study of digestibility and nutrients in herbaceous foliage from African montane vegetation (Waterman et al. 1983). These plants were analysed because of their inclusion in gorilla diet. In this chapter, I present results of a stepwise regression analysis of eighteen plant chemical characteristics, both deterrent and beneficial, on antelope browse preference.

5.3 METHODS

A data matrix was assembled consisting of 65 rows (plant species) and 19 columns (18 plant chemical characteristics, and one measure of browse) using data derived from three sources. Twelve columns comprised data for different polyphenol assays (Chapter 5.2, Appendix 5.2.1). Each of four assays was performed on a pure and an aqueous methanol extract from each plant species. The results from the two extracts were summed to give a total for each plant species for each test, thus creating a third column for each test (e.g. polyphenol A, polyphenol B and polyphenol total).

A further six columns comprised data from nutritional analyses in the following catagories: protein, carbohydrates (nitrogen-free extract or NFE), fats (ether extract), crude fibre, moisture content and ash. All six were determined by proximate or Weende analysis (Van Soest 1983). The nineteenth column comprised browse preference or Jacobs D indices (Jacobs 1974) derived from trail transects (Section 5.1, Table 5.1.1, Appendix 5.1.3). The 18 columns of polyphenol and nutritional data were arcsin transformed to improve their distributional characteristics for multiple regression analysis (Zar 1974). The 65 row entries comprised data determined for15 plant species during each of four seasons.

The matrix was analysed using a multiple linear regression and stepwise multiple regression analysis program (Draper & Smith 1981). This computer program was available on STATPRO which was used on an Apple II microcomputer.

5.3 RESULTS

Stepwise analysis of browse, polyphenols and nutrition

Multiple regression analysis of the plant chemical and nutritional data as the independent variables, and Jacobs D index of browse as a dependent variable indicated that higher pairwise correlations were found between browse and measures of tannin polyphenols than with the measures of nutrition (Table 5.3.1). All tannin polyphenol measures were inversely correlated with browse. Total polyphenols, total astringency and total polyphenols in aqueous methanol extracts had the highest pairwise coefficients (r = -0.34). Stepwise analysis of this data matrix indicated that total polyphenols contribute significantly to the overall regression as Step 1 (Table 5.3.2) and condensed tannins measured on pure methanol extracts were significant Step 2 contributors to the regression.

A subset of the whole matrix comprising plants collected during the dry season was also examined using multiple regression analysis. The correlation matrix was substantially the same as that for the entire data set (Table 5.3.3). However, the pairwise coefficients for browse and each of the other independent variables were somewhat higher (Table 5.3.2).

It should be noted here that a significant F statistic does not necessarily imply that the independent variable in question is a good predictor of the dependent variable. There is evidence that the F value for a given predictor should be at least four times greater than the tabulated F value for the chosen significance level to be regarded as a good predictor in the overall regression (Draper & Smith 1981).

In the stepwise analysis, the best independent variable predictors for browse during the dry season are different from those for browse during both seasons combined (Table 5.3.2). These were: astringency in pure methanol plant extracts, followed by proanthocyanidins in aqueous methanol and in pure methanol extracts respectively.

Seasonal changes in nutritional components

Differences from season to season in the six nutritional components from proximate analysis were minimal (Table 5.3.4): in most cases the differences were smaller than the standard deviations. Protein levels increased from 6 % in summer to 8 % on autumn then declined to 7 % through winter to spring. Fibre was lowest in winter (17 %) and increased through summer to an autumn high of above 19 %. Similarly, carbohydrates (NFE) were low in spring (52 %) and increased to a winter high of 54 %. Moisture content was also lowest in spring (58 %), increasing to a winter high of 62 %. Ash content was marginally higher in the wet season , while fats (ether extract) fluctuated around 6 %.

A comparison of all dietary components in wet and dry seasons indicated that there were significant differences in some of the polyphenol tests but in none of the other nutritional components (Table 5.3.5).

5.3 DISCUSSION

In this discussion, supporting evidence from other vertebrate herbivory studies is

reviewed. This is followed by an examination of the chemical interaction of some cellular components during digestion, which serves as background for an outline of some of the size limitations in digestion for small ruminants.

Polyphenols and preference

The various measures of polyphenol levels obtained in this study were the strongest predictors of browse patterns, while nutritional variables did not contribute significantly to a predictive equation. Secondly, there is evidence for a seasonally discrete pattern: data from the dry season provided a much higher R^2 than those from the four seasons combined. Inadequate sample sizes for winter meant that it was not possible to assemble a complete matrix for analysing effects in the wet season. The results of this analysis are encouraging given the complex chain of events from leaf chemical composition on the one hand to evidence of browse on the other, and the likelihood that the relationship between the two will be non - linear(Westoby 1974).

The results reported here are, to my knowledge, the first for an African ruminant in a low-nutrient, vegetationally diverse ecosystem. A number of other studies, mainly in low-nutrient tropical ecosystems, support this finding. In a review of browsing on subarctic vegetation, Bryant and Kuropat (1980) concluded that vertebrates avoided plant tissues containing high concentrations of secondary chemical constituents and that proximal nutrient content was inconsequent for this selection. The secondary chemicals in question were mostly digestibility-reducing resins. These preference patterns are also found in domesticated animals in managed systems. Deer and sheep (Cooper-Driver et al. 1977) and steers (Donnelly 1954) all showed preferences for low tannin plants while astringency depressed food intake in rats(Tamir & Alumot 1970, Glick & Joslyn

1970a) and ruminants (Arnold & Hill 1972, Burns et al. 1972, Burns & Cope 1974).

The food choices of primates are particularly interesting because these animals are all highly mobile and able to sample a wide species diversity of plant material. Colobus guereza in Kibale, Uganda preferred leaf material which had high protein, low lignin, low crude fibre and low tannins, while alkaloids were unimportant (Struhsaker 1975, Oates et al. 1977). Red colobus C. bodius , more frugivorous than C. guereza , selected leaf petioles and ignored the leaf blade. The petioles were found to have lower total phenolics, condensed tannin and higher acid detergent fibre (Struhsaker 1975, 1978). Black colobus C. satanus in Douala-Edea forest in the Cameroon strongly preferred seeds and avoided the mature leaves of the 15 most common tree species, in contrast with the colobines mentioned above. A ratio of nitrogen content : (acid detergent fibre + condensed tannins) was significantly correlated with choice (Gartlan et al. 1980). The Cameroon forest occurred on a low nutrient white sand substrate with low levels of soil nitrogen and the leaf material had higher concentrations of tannins and lower alkaloid levels than Kibale forest in Uganda (McKey et al. 1978). Howler monkeys Alouatta palliata in Costa Rica, used only 20% of the trees in their habitat for food (Glander 1978). They actively avoided condensed tannins, alkaloids and high fibre and selected plant material with high protein and balanced amino-acids (Glander 1978, Milton 1979). In central Africa, chimpanzees ate fruits which contained lower levels of condensed tannins when unripe compared with other available fruits. These ripe fruits also had greatly reduced levels of extractible condensed tannins than the unripe fruit (Wrangham & Waterman 1983). Food items in the diet of rhesus monkey Macaca mulatta in the Himalayan foothills, contained low tannins and most of the essential amino acids. However protein, water, fibre, lignin and alkaloids did not influence food choice (Marks 1985).

Distinction between different dietary components

Animals require specific combinations and concentrations of nutritional compounds for survival, growth and reproduction (Robbins 1983): preference behaviour permits a range of adjustment for specific requirements. However, all leaf material contains proportions of compounds which are not directly catabolisable(e.g. cellulose), and also the proportions of beneficial nutrients are surprisingly constant in many species which means that the range of adjustment is constrained. Fraenkel (1959) pointed out in a land-mark paper that the leaves of most plants have similar nutritional value and it is the secondary metabolites which influence herbivore food choice. In the present study, the small increase in protein through summer and autumn was offset by the much larger increase in polyphenol levels. This is interesting in relation to browse levels in the vegetation (Figure 5.1.4) which showed a decline during the same period. Apart from avoiding secondary compounds, it seems that animals will make changes in their diet according to metabolic needs as well. Topi Damaliscus korrigum in Serengeti, for example, selected mature or dry grass with higher proportional protein over green grass to increase the protein quality of the diet (Jarman & Sinclair 1979). Impala Aepyceros melampus shift from grazing to browsing if the water content of forage falls below 30% and they also need to drink additional water (Jarman & Sinclair 1979).

Both the improved chemical definitions of nutritional components and the determination of animal requirements, particularly those of herbivores, have led to improvements in analysing the interactions between food quality and dietary choice. This has meant re-examining dietary physiological requirements using a combination of experimental and ecological approaches (Robbins 1983, Van Soest 1983) on the one hand, and redefining different dietary categories e.g. fibre and nitrogen on the other.

A complex of lignins and polysaccharides, described below, are commonly measured together as "fibre". The evidence suggests that there are clear differences in the digestion of each and that significant and antagonistic effects occur between components. Nutritional research during the past two decades has questioned the validity of Weende and proximate analysis, particularly in its concept and determination of crude fibre (Van Soest 1983). In Weende or proximate analysis, 'crude fibre' includes everything remaining after other components have been extracted. One of those remaining is cellulose. Cellulose, the most abundant compound in nature (Van Soest 1983), comprises the cell wall in plants, together with hemicellulose and lignin. This complex is impervious to breakdown by hydrolytic enzymes from the digestive tracts of animals. Certain micro-organisms do excrete cellulolytic enzymes in the ruminant fore-stomach or in the caecum of hindgut fermenting animals and can degrade these structural carbohydrates in fermentation and supply energy in the form of volatile fatty acids as well as other essential trace compounds (Robbins 1983). Cell wall constituents may inhibit the extraction of other components from inside the cell (Janis 1976), although Van Soest (1983) cites results of balance studies using different forage compositions which suggest that digestion of cell contents is unaffected by the presence of cell wall. Cell walls also continue to have the same effective volume until they are broken down by fermentation (Van Soest 1983). Caecal fermenters digest less cellulose and hemicellulose than ruminants, although digestion of these components has been noted for horses (Janis 1976), voles (Van Soest 1983) and howler monkeys (Milton et al. 1980). Lignins (as distinct from lignified cell wall material) also interfere with the digestion process. Since the efficacy of proximate analysis in determining nutritional components is questionable, this may provide a partial explanation for the apparent lack of effect of 'beneficial' nutritional compounds in determining antelope preference.

With the developement of acid- and neutral-detergent(AD and ND) analysis methods (Goering & Van Soest 1970, Van Soest 1983) a more biologically meaningful definition of fibre now exists. Detergent analyses are an improvement but all problems have not been solved, particularly with regard to secondary metabolites (Robbins 1983). Tannin-protein complexes will reduce apparent digestibility of plant components which are soluble in neutral detergent, while neutral detergent solubles of grasses and plants with minimal concentrations of inhibitory secondary compounds are 98 - 100% digestible in all herbivores (Van Soest 1967). The insolubility of tannin complexes in ND and AD interferes specifically in predicting cell wall digestibility and dry matter digestibility from feed intake (Robbins 1983). For example, cell wall digestibility was reduced by the addition of hydrolysable tannin (as tannic acid) to an in vitro rumen fermentation (Horvath 1981). The addition of protein to the system decreased the degree of tannic acid inhibition. However, some tightly bound tannic acid appeared to inhibit cell wall digestion and could not be removed by protein addition (Horvath 1981). In another study of digestibility, condensed tannins from 40 browse plants in the diet of African kudu were found to inhibit in vitro fermentation of lucerne (Van Hoven 1984). A hydrolysable tannin (tannic acid) had a similar effect on fermentation.

The protein value in this study, as with most other studies of this type, was derived from a nitrogen analysis and converted using a standard value, usually 6.25 (Robbins 1983). This value is derived from the ratio of elemental nitrogen in protein molecules (approximately 16 %), and can be higher or lower depending on the amino acid composition of the proteins under consideration and on the presence of other N containing compounds. For example, the content of protein from spinach, broad beans, alfalfa, cabbage and corn leaves converts accurately with a factor of 6.49 ± 0.51 (Van Soest 1983). On the other hand, in a study of the protein composition of some tropical leaf, fruit and flower samples, Milton and Dintzis (1981) found that a conversion of 4.4

± 0.5 was more realistic. These two examples suggest that protein content should be determined in each vegetation type. Ideally, this should be supplemented with amino-acid analyses of the plants being studied. Cellulose and nitrogen represent two of at least six major nutritional categories; during digestion polyphenol types of compounds clearly have complex chemical interactions with both of these.

Secondary compounds and digestion in ruminants

Plant phenolic compounds inhibit many different types of digestive enzymes : cellulase and pectinase (Bell at al 1963), beta - glucosidase (Goldstein & Swain 1965, Loomis & Battaile 1966), peroxidase, catalase and alcohol dehydrogenase (Goldstein & Swain 1965), trypsin (Feeny 1969, Chavan et al 1979), amylase and lyase (Milic et al. 1972). However, there is some evidence that this inhibition can be offset in some animals by physiological adjustment in the duodenum, intestine or caecum. In rats, a 5% tannin diet increased intestinal proteolytic activity 3 - 4 fold (Glick & Joslyn 1970b) while carob tannin increased trypsin activity in the caecum (Tamir & Alumot 1970). Field bean tannins increased pancreatic secretion in rats (Griffiths & Moseley 1980) but trypsin and alpha - amylase levels decreased. Invertebrates, specifically lepidopteran larvae, have gut pH levels adjusted to a higher level (Feeny 1970). Subsequently Berenbaum(1980) assembled inferential evidence for elevated gut pH related to the presence of tannins in the dietary plants of 60 lepidopteran species. There appears to be very little evidence about the effects of secondary metabolites on the digestive tract micro-organisms, apart from some differential effects of terpenoids. Cellulolytic rumen bacteria are more susceptible to terpenoid toxicity than starch-digesting bacteria (Oh et al. 1967, Schwartz et al. 1980).

A physiological effect of polyphenolic intake in mammals is that fecal N increases above

levels seen on non-phenolic forage (Mould & Robbins 1981). For example, soluble phenolics of fireweed Epilobium angustifolium , and maple leaves reduced the apparent digestibility of protein when fed to elk. This also was associated with increases in metabolic fecal nitrogen (MFN) excretion (Mould & Robbins 1982). A partial physiological explanation for this phenomenon of elevated MFN excretion is as follows : more N passes out of the rumen than enters it when tannins are present in the diet(MacRae et al. 1979). This may be because the N in protein is unavailable to the rumen microrganisms because it is complexed with the tannins. As a result, when available nitrogen levels in the diet fall below the the microbial requirement, plasma urea may diffuse into the rumen. This diffusion is triggered by decreased ammonia production due to a decline in microbial activity (Egan & Ulyatt 1980). The urea is hydrolysed into ammonia which the microbial population can then use for protein synthesis. Once the digestion contents move post - gastrically and depending on the type of tannin, some ruminants may then be able to digest the protein part of the tannin - polyphenol complex. This is because the pH increases post - gastrically and this may dissociate the complex. In small ruminants there is more metabolic fecal N excretion because of more rapid rumen or lower-tract fermentation and larger gut surface area to volume ratio which increases bacterial protein and thus MFN excretion.

Finally, there is some evidence that tannins in the diet can beneficially prevent the development of bloat in some animals, including colobine monkeys with their fore-stomach fermentation (Marks 1985) and ruminants (Jones et al. 1976, 1973).

Digestion and limitations in rumen size of small herbivores

The small shrubland-dwelling antelope which were the subject of this study have had to cope with a diet containing both low levels of nitrogen and higher tannin polyphenols

with the particular physiological implications of this combination. They appeared to have adapted to this by having browse preference behaviour which is highly selective. However because of their size, it is also necessary to consider some size - specific physiological processes. In all animals, both protein and energy requirements (per kg body weight) increase with decreasing body size. Grazing herbivores are limited by the size of the rumen and by diet quality, and there are few grazers below 70 kg body weight (Van Soest 1983). In ruminants, non-ruminants, hind-gut fermentors and fore-gut fermentors, gastrointestinal capacity, calculated from a log of the wet weight of fermentation contents, has a log relation with body weight. Small ruminants have a small fermentation capacity ($Y = 0.1050\ X^{1.05}$, where X = body weight (kg) and Y = fermentation contents per unit of energy requirement calculated as ($W^{0.75}$)). Because of this, high selectivity becomes necessary when poor quality forage is all that is available. Rumination then has to increase to achieve sufficient intake for more than maintenance requirements. Small ruminants will reach an intake limit before larger ones (Van Soest 1983), and it follows that they have a faster passage rate. Because of this, fibre is not digested to the same degree. The smaller rumen capacities of browsers and selective feeders appear to be associated with a more digestible diet. Such a diet, although it has a high lignin content, also has a higher digestible cellular content. Ruminants and large non-ruminant herbivores digest more of the neutral detergent fibre (NDF) than do small non-ruminant herbivores. Small non-ruminant herbivores digest only 20% of NDF and passage rate is too fast for available cellulose and hemicellulose to be used; structural inhibitors have no effect. In general, ruminants are not all equal in ability to digest fibre (Hungate et al. 1959, Prins & Geelen 1971, Short et al. 1974 (in Van Soest 1983)) : the differences are attributable to rumen capacity and gastrointestinal passage phenomena (Van Soest 1983). The ability of any particular ruminant to digest fibre is very difficult to test experimentally (Van Soest 1983).

In conclusion, the results of this study indicate that small antelope make dietary choices for individual plant species particularly to avoid high levels of tannin polyphenols rather than to obtain some beneficial nutritional compound. This finding agrees with similar studies on herbivorous vertebrates in other low-nutrient ecosystems. A short review of the literature highlights some of the undisputable physiological effects of tannin polyphenols, while also emphasizing that many of the unanswered questions about digestion are related to the effects of these and other secondary metabolites specifically on the process of digestion.

	Poly A	Poly B	Poly Tot	Haem A	Haem B	Haem Tot	Pro A	Pro B	Pro Tot	Flav A	Flav B	Flav Tot	Protein	Fibre	Fats	Carbo	Met ener	Moist	Ash
Poly B	0.35																		
Poly Tot	0.97	0.57																	
Haem A	0.79	0.40	0.79																
Haem B	0.01	0.50	0.14	0.15															
Haem Tot	0.75	0.49	0.78	0.97	0.37														
Pro A	0.78	0.13	0.72	0.75	-0.02	-.69													
Pro B	0.44	0.38	0.48	0.35	0.08	0.35	0.50												
Pro Tot	0.78	0.16	0.73	0.75	-0.02	0.69	0.99	0.58											
Flav A	0.78	0.13	0.72	0.71	-0.18	0.62	0.85	0.53	0.85										
Flav B	0.50	0.48	0.54	0.46	0.15	0.45	0.45	0.76	0.50	0.51									
Flav Tot	0.78	0.17	0.73	0.72	-0.16	0.64	0.81	0.50	0.82	0.97	0.55								
Protein	-0.02	0.27	0.07	0.07	0.24	0.13	-0.24	-0.33	-0.26	-0.24	-0.16	-0.18							
Fibre	-0.23	-0.47	-0.34	-0.28	-0.47	-0.37	-0.29	-0.09	-0.28	-0.07	-0.16	-0.05	0.01						
Fats	0.28	-0.01	0.26	0.40	0.23	0.44	0.17	0.08	0.17	0.31	0.03	0.33	0.04	-0.02					
Carbohyd	0.08	0.22	0.11	0.02	0.04	0.01	0.28	0.32	0.30	0.12	0.37	0.10	-0.46	-0.59	-0.49				
Met energy	0.29	0.06	0.28	0.36	0.16	0.39	0.40	0.37	0.42	0.44	0.24	0.46	-0.31	-0.31	0.61	0.22			
Moisture	0.21	0.30	0.29	0.19	0.47	0.30	0.03	-0.15	0.01	-0.05	-0.22	-0.08	0.46	-0.48	0.25	-0.27	-0.04		
Ash	0.10	0.39	0.21	0.10	0.49	0.22	-0.08	-0.22	-0.10	-0.21	-0.20	-0.25	0.36	-0.60	0.09	-0.11	-0.19	0.89	
Browse	-0.28	-0.34	-0.34	-0.33	-0.05	-0.34	-0.06	-0.27	-0.09	-0.10	-0.30	-0.11	-0.09	0.10	-0.10	0.04	-0.03	-0.20	-0.15

Table 5.3.1 Matrix of partial correlation coefficients analysing the correlation between 18 independent variables and observed browse patterns covering four seasons in shrubland vegetation.

Table 5.3.2 Results of stepwise multiple regression analysis on data from Tables 5.3.1 and 5.3.2. For browse from all seasons $df_1 = 19$, $df_2 = 17$, $F_{0.05} = 2.31$, $F_{0.01}=3.31$. For browse from the dry season only $df_1 = 19$, $df_2 = 7$, $F_{0.05} =3.51$, $F_{0.01} = 6.31$.

Dependent variable	F	Partial F	r	R^2	SD of residuals	SE as % of means	B coeff
Browse (all seasons)							
Step 1 Total polyphenols (100% + 50 % MeOH)	4.68	7.74	-0.34	0.12	0.51	79.8	-0.06
Step 2 Pro-anthocyanidins (100 % MeOH)	3.95	2.96	-0.06	0.19	0.51	77.6	0.33
Constant							1.40
Browse (dry season)							
Step 1 Astringency (100% MeOH)	7.37	15.09	-0.47	0.22	0.50	72.3	-0.07
Step 2 Pro-anthocyanidins (50% MeOH)	5.78	9.33	-0.43	0.32	0.47	69.1	-3.64
Step 3 Pro-anthocyanidins (100%MeOH)	8.05	8.93	-0.14	0.50	0.41	60.2	0.57
Constant							1.70

	Poly A	Poly B	Poly Tot	Haem A	Haem B	Haem Tot	Pro A	Pro B	Pro Tot	Flav A	Flav B	Flav Tot	Protein	Fibre	Fats	Carbo	Met ener	Moist	Ash
Poly B	0.41																		
Poly Tot	0.97	0.60																	
Haem A	0.76	0.42	0.76																
Haem B	0.10	0.47	0.21	0.24															
Haem Tot	0.74	0.48	0.76	0.98	0.42														
Pro A	0.77	0.17	0.71	0.73	0.12	0.71													
Pro B	0.39	0.46	0.46	0.29	0.21	0.32	0.44												
Pro Tot	0.77	0.21	0.72	0.73	0.14	0.70	0.99	0.52											
Flav A	0.76	0.15	0.70	0.68	-0.08	0.62	0.83	0.47	0.84										
Flav B	0.51	0.60	0.57	0.45	0.29	0.47	0.43	0.77	0.49	0.52									
Flav Tot	0.77	0.20	0.72	0.69	-0.07	0.63	0.79	0.44	0.80	0.97	0.55								
Protein	-0.05	0.07	-0.03	0.04	0.03	0.04	-0.25	-0.34	-0.27	-0.23	-0.17	-0.16							
Fibre	-0.26	-0.47	-0.36	-0.30	-0.50	-0.37	-0.37	-0.12	-0.36	-0.11	-0.17	-0.07	0.23						
Fat	0.36	-0.02	0.33	0.45	0.23	0.48	0.22	0.08	0.22	0.37	0.05	0.38	0.09	-0.03					
Carbohyd	0.02	0.29	0.08	-0.02	0.17	0.01	0.27	0.32	0.29	0.10	0.33	0.07	-0.60	-0.61	-0.50				
Met energy	0.31	0.05	0.29	0.36	0.20	0.38	0.44	0.38	0.46	0.49	0.22	0.50	-0.44	-0.30	0.59	0.24			
Moisture	0.33	0.17	0.35	0.24	0.31	0.29	0.12	-0.17	0.09	0.00	-0.19	-0.04	0.31	-0.44	0.33	-0.30	-0.05		
Ash	0.15	0.32	0.23	0.13	0.41	0.20	-0.05	-0.24	-0.07	-0.23	-0.19	-0.26	0.25	-0.53	0.14	-0.17	-0.23	0.88	
Browse	-0.41	-0.38	-0.45	-0.47	-0.01	-0.45	-0.14	-0.43	-0.18	-0.23	-0.40	-0.22	0.05	0.00	-0.29	0.15	-0.11	-0.25	-0.08

Table 5.3.3 Matrix of partial correlation coefficients analysing the correlation between 18 independent variables and observed browse pattrens during the dry season (Summer and Autumn) in shrubland vegetation.

Table 5.3.4 The results (% dw) of proximate analysis on a selection of shrubland plant species which were collected seasonally. Data from Appendix 5.3.1.

	Summer			Autumn			Winter			Spring		
	n	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.
Protein	15	5.9	0.3	20	7.9	0.9	11	7.5	1.3	19	7.1	0.6
Fibre	15	19.1	1.6	20	19.2	1.9	11	16.9	2.2	19	18.0	2.0
Ether extract (Fat)	15	6.2	1.1	20	6.4	0.9	11	6.0	1.0	19	6.4	0.8
NFE extract (Carbohydr.)	14	52.5	2.1	19	52.9	2.3	10	54.1	2.3	19	51.8	2.2
Moisture	14	58.6	4.0	19	61.3	3.6	10	61.7	7.0	19	57.6	3.9
Ash	14	8.0	1.0	19	8.0	1.3	10	8.2	1.3	19	8.2	0.9

Table 5.3.5 Comparison of polyphenol and nutritional components (Mean and S.E.) of strandveld plants in wet and dry seasons. Polyphenol data from Appendix 5.2.1 and nutritional data from Appendix 5.3.1. (A = 100 % MeOH extract;B = 50% MeOH extract; Tot = A + B ;NS = not significant).

	Wet season			Dry season			
	$\bar{x}$	S.E.	H_0	$\bar{x}$	S.E.	t	p
Polyphenols							
A	3.67	0.52	<	8.16	1.08	3.75	< 0.001
B	1.66	0.23	<	1.92	0.27	0.72	> 0.1(NS)
Tot	5.32	0.64	<	10.11	1.20	3.53	< 0.001
Haemanalysis							
A	4.24	0.56	<	8.24	1.06	3.34	< 0.01
B	1.59	0.33	>	1.47	0.23	0.30	> 0.1(NS)
Tot	5.79	0.72	<	9.68	1.15	2.87	< 0.01
Proanthocyanidins							
A	18.23	5.21	<	56.35	10.56	3.24	< 0.01
B	4.67	1.02	<	6.44	1.21	1.11	> 0.1(NS)
Tot	22.77	5.90	<	62.76	11.16	3.17	< 0.01
Flavanols							
A	5.85	1.69	<	16.30	3.30	2.82	< 0.01
B	0.55	0.13	<	1.10	0.20	2.28	< 0.05
Tot	6.36	1.73	<	17.37	3.40	2.89	< 0.01
Protein	7.20	0.59	>	7.08	0.55	0.12	> 0.1(NS)
Crude Fibre	17.60	1.49	<	19.16	1.25	0.80	> 0.1(NS)
Ether Extract	6.27	0.61	<	6.30	0.68	0.03	> 0.1(NS)
NFE	52.57	1.61	<	52.73	1.54	0.07	> 0.1(NS)
Moisture	59.02	3.47	<	60.16	2.64	0.26	> 0.1(NS)
Ash	8.19	0.72	>	8.04	0.83	0.14	> 0.1(NS)

Appendix 5.3.1. Results (% d.w., except for moisture) of proximate analyses performed on selected shrubland species which were collected seasonally. The five dry weight values usually total up to less than 100 % because of reabsorbing atmospheric moisture after the initial drying and water content determination.

	Protein	Fibre	Ether Ex	NFE	Moisture	Ash
SUMMER						
Chrysanthemoides incana	7.4	15.7	2.8	53.9	74.5	11.1
Colpoon compressum	5.0	8.4	2.3	69.1	57.8	6.9
Eriocephalus africanus	8.5	18.5	6.3	44.6	69.8	13.6
Euclea racemosa	6.1	25.5	8.4	46.1	52.9	6.3
Euphorbia burmannii &E. caput-medusae	4.9	14.0	15.2	43.9	88.2	13.1
Nylandtia spinosa	5.0	22.6	1.6	62.3	30.0	3.1
Olea exasperata	5.8	26.1	9.1	47.0	43.2	5.1
Passerina vulgaris	5.8	30.1	10.4	43.0	46.6	4.4
Pterocelastrus tricuspidatus	5.5	14.0	4.1	58.3	64.7	8.7
Putterlickia pyracantha	6.5	15.0	3.0	56.3	63.9	9.7
Rhus incana	6.6	20.9	3.9	54.6	44.8	6.1
Rhus lucida	5.4	12.1	12.5	55.6	60.9	5.7
Salvia aurea & S. nivea	6.4	15.4	8.6	46.7	70.7	14.6
Thesium aggregatum	5.8	20.4	2.9	-	-	-
Willdenowia striata	4.3	28.0	1.4	53.8	52.5	4.0
AUTUMN						
Aspalathus hispida	9.7	24.7	6.3	52.5	43.3	3.0
Asparagus capensis	17.1	37.3	4.8	29.9	65.4	7.1
C. incana	8.3	15.6	3.3	60.2	66.6	8.2
C. compressum	8.0	29.2	11.3	43.1	61.8	4.0
Cotyledon orbiculata	4.1	6.3	4.0	64.4	82.3	14.3
E. africanus	12.7	18.3	8.1	44.1	76.3	11.7
E. racemosa	5.1	23.8	10.2	52.2	51.7	5.3
E. burmannii & caput-medusae	5.3	14.9	16.2	47.7	84.8	11.2
Monochlamys sp.	17.0	10.7	3.1	39.0	85.7	25.8
N. spinosa	5.6	25.6	6.9	57.1	25.5	1.6
P. vulgaris	4.7	9.4	3.2	72.1	56.6	6.9
Phylica stipularis	5.5	27.0	10.3	50.0	44.3	3.3
P. tricuspidatus	5.9	15.0	5.4	61.2	62.6	8.4
P. pyracantha (hard stem)	6.5	13.7	3.1	60.3	66.3	7.6
(soft stem)	12.0	8.5	1.7	-	-	-
R. incana (old)	6.1	19.3	6.3	56.6	50.0	6.1
R. lucida	5.6	14.1	10.8	59.1	56.5	5.3
S. aurea & nivea	11.0	15.6	9.0	45.7	75.3	10.2
T. aggregatum	3.8	24.3	2.1	55.6	60.9	9.5
W. striata	4.8	30.6	2.0	54.2	48.8	3.4
WINTER						
C. incana	8.2	12.4	3.7	58.6	79.0	12.9
C. compressum	4.9	7.9	2.9	62.7	61.5	11.3
E. africanus	13.8	16.0	5.0	47.8	87.6	12.0
E. burmannii & caput-medusae	5.7	17.7	13.8	43.0	83.4	10.1

cont.

Appendix 5.3.1 cont.

N. spinosa	5.9	21.1	6.9	57.2	19.7	2.3
Protea nereifolia	2.7	24.0	4.6	61.0	53.7	2.3
P. tricuspidatus	4.0	13.2	6.1	58.9	59.3	10.1
R. lucida (old)	6.1	13.2	9.4	56.7	48.2	5.4
S. aurea & nivea	14.9	14.9	6.3	47.4	83.9	11.5
Senecio elegans	11.8	11.0	5.3	-	-	-
SPRING						
C. incana (new)	8.3	17.7	2.4	55.0	70.5	8.7
(old)	5.4	13.8	2.9	59.7	57.1	9.4
C. compressum (new)	5.7	9.5	2.4	67.0	60.5	7.3
(old)	4.0	9.1	3.9	61.8	69.1	12.0
E. africanus	8.6	17.1	6.7	44.7	72.0	13.2
E. racemosa (new)	7.7	14.9	5.0	59.3	65.5	5.2
(old)	4.6	25.3	9.6	46.8	50.2	6.0
E. burmannii & caput-medusae	4.8	1.3	12.2	59.4	86.5	12.4
Haemanthus sp.	13.4	24.8	6.7	27.4	86.6	17.1
N. spinosa	7.5	22.9	8.3	51.7	34.9	2.7
O. exasperata (new)	11.4	21.9	8.6	44.4	56.3	6.7
(old)	5.7	24.6	8.4	47.1	35.2	8.2
P. vulgaris	6.1	32.4	10.5	39.6	52.8	4.0
P. tricuspidatus (new)	8.0	10.8	2.5	62.0	67.6	6.7
(old)	4.1	12.4	6.0	57.8	47.7	9.9
R. incana (old)	10.2	17.5	3.4	53.2	43.5	6.1
R. lucida (old)	5.8	12.9	12.4	54.4	51.8	5.4
S. aurea & nivea	8.4	15.1	8.6	46.6	67.4	11.8
W. striata	4.3	38.4	1.6	45.7	20.4	2.7

5.4 BIOMASS AND PRODUCTION OF STRANDVELD VEGETATION

5.4 INTRODUCTION

The particular eco-physiological constraints on plants growing in mediterranean ecosystems in general (Mooney & Gulmon 1982) suggest that the vegetation is nutritionally marginal for both vertebrate and invertebrate herbivores (Mattson 1980). As part of the study dealing with plant species choice by small antelope and the biochemical and nutritional profile of the food plants (Chapters 5.1, 5.2 and 5.3), it was considered necessary to determine that antelope, although occurring at low densities, were consuming measurable amounts of plant biomass. Generally, it is possible to obtain good estimates of consumption by grazing in physiognomically homogeneous grasslands by measuring grass height before and after grazing and then using appropriate formulae to calculate biomass removed (Barnes 1976 and references therein). However, browse is often much more difficult to measure, a fundamental problem being the high spatial and temporal variability of browse occurrence (Bobek & Dziecidowski 1972 in Rutherford 1979). Exclusion plots are a commonly used method of estimating vegetation consumption by direct difference and were considered more appropriate for this mediterranean shrubland with its very diverse physiognomy. The questions addressed in this study were: 1) what is the standing crop of plant biomass and the productivity of strandveld shrubland; and 2) how do these values compare with other mediterranean- and low-nutrient ecosystems?

5.4 METHODS

Study site and exclusion plot construction

Four 15m x 15m exclusion plots, randomly located in a relatively homogeneous 12 ha

strip of vegetation (Figure 5.4.1), were erected in October 1979. The plots were harvested after nine months at the end of June 1980. The fencing material used for the plots consisted of a 15m strip of 40% shade netting with approximately 2mm mesh size (Alnet, Epping Industria, Cape Town). The netting was suspended vertically on 8mm diameter reinforcing rods which were driven into the sandy substrate and staked to the sand with 1m semicircular hoops (Figure 5.4.2). The vertical rods were placed 3m apart for optimal support during the seasonally prevailing strong winds. The plots were checked and maintained regularly.

Initial biomass determination

Initially, seven randomly placed 1 m^2 quadrats of above-ground biomass were clipped in the vicinity of, but outside, each of the four exclusion plots. Each quadrat sample was weighed, sorted into photosynthetic (leaves and soft twigs) and non-photosynthetic (woody) material and the photosynthetic material was then weighed separately. The mass of the woody material was taken to be the difference between the total mass and the mass of the photosynthetic material. Representative sub-samples were taken from both woody and leaf material samples from each of the four exclusion plots. These were oven-dried to constant mass at 60°C to determine the moisture content of the fresh material. These initial above-ground biomass values provided a basis for estimating production.

Final biomass determination

At the conclusion of the experiment 22 1m^2 quadrats were located randomly both inside and surrounding the outside of each of the four exclusion plot experiments respectively. A set of random coordinates was used to choose 22 out of a possible 225 quadrats inside the plot

(Zar 1974). This ensured that at least 10% of the ground area was sampled within each exclusion plot. Each quadrat was clipped and the plant material separated into the same categories and weighed as for the initial biomass estimate. The sorted phytomass sample from the 22 quadrats was then remixed and a sub-sample taken, weighed in the laboratory and oven-dried to constant mass at 60°C. The woody complement was treated in the same way. The wet : dry weight ratios were used to convert all wet weight estimates to dry weight. The dry phytomass value was added to the woody dry weight in order to obtain an estimate for total biomass.

Antelope in the study area

Three species of antelope were present in the study area in which the exclusion plots were located (C Duckitt, personal communication). These were grey duiker Sylvicapra grimmia (Cephalophinae), steenbok Raphicerus campestris and grysbok R. melanotis (Neotraginae). The scope of other research conducted simultaneously with this project made it impractical to conduct a full-scale estimate of population density. Nevertheless it was considered important to determine that antelope were present in the study area surrounding the exclusion plots. The existing stock fence around the perimeter of the study area appeared to be no barrier to their movement (personal observations). Each month between February 1979 and May 1980, two observers on horseback rode five set transects accompanied by a trained dog. The observers moved at walking pace parallel to each other at approximately 30m distance while the dog ranged between them. The transects were covered at dawn and at dusk in random order. All sightings were noted on a map drawn from an aerial photograph of the study site, which had been divided into 1 ha blocks (Figure 5.4.1). The cryptic coloration and predator avoidance behaviour of these antelope induced "detection dependent on observer" factors (Eberhardt 1978).

These factors introduce complexities into population estimation models which are very difficult to resolve for accurate density estimations (Eberhardt ibid.).

5.4 RESULTS

Biomass estimate

At the end of 9 months, the total biomass outside the exclusion plots was significantly lower than the biomass inside (Table 5.4.1) (but see addendum on back cover). The difference in the phytomass was 25% while the woody material showed a surprisingly high 45% decrease. Wood comprised about 67% of the total biomass and the high level of significance ($p < 0.005$) of the difference in total biomass means can probably be attributed to this.

The large standard deviations made it advisable to calculate asymptotic confidence intervals on the differences in the means using the standard error incorporated into the following formula, based on the central limit theorem:

$$\bar{x}_1 - \bar{x}_2 \pm \sqrt{SE_1^2 + SE_2^2} \cdot 1.96$$

(for confidence intervals at the 95% level).

The limits calculated from this formula should be greater than zero in order to have full confidence in the calculated t-statistics (Table 5.4.2) (L. Mueller, pers. comm.). The limits for wood and total biomass are both greater than zero but the lower limit for phytomass is negative. The large sample size and the small magnitude of the negative value made it seem reasonable to assume that the difference in the means was a meaningful reflection of an observable biological phenomenon. The biomass value obtained in this study was compared with published values from other mediterranean

ecosystems (Table 5.4.3) and these values are discussed below.

Production estimate

A production value of 360 g m^{-2} yr^{-1} was estimated for strandveld vegetation. It was calculated fairly crudely by obtaining the difference between means for vegetation samples clipped before the exclusion plots were set in place(1170 ± 730 g m^{-2} ; n =28) and means for samples from inside the plot at the conclusion of the experiment(1440 ± 1560 g m^{-2} ; n = 88). The value obtained (270 g m^{-2}) accounted for production during a nine month period and was adjusted accordingly to represent an annual estimate. This was compared with published estimates for primary production in some other mediterranean-type ecosystems as well as some values for South African grasslands(Table 5.4.4).

5.4 DISCUSSION

Biomass estimates for above-ground plant material in mediterranean-type vegetation range from 660 g m^{-2} for South Africa up to 2700 g m^{-2} for Australian evergreen heath which was unburnt for 25 years. Biomass is extremely low in all mediterranean-type ecosystems (Table 5.4.3) compared with Australian Eucalyptus and temperate deciduous forest and this is certainly due to the low soil fertility which is characteristic of these ecosystems (Table 4.0.1). These values are between 6 and 20 times lower than those for a small sample of temperate ecosystems on higher nutrient soils (Table 5.4.5). Biomass of a semi-arid montane woodland is six times higher than the highest value for mediterranean vegetation, while mixed lowland forest comprises 20 times more biomass per unit area.

Production estimates for mediterranean ecosystems (Table 5.4.4) are also extremely low relative to other ecosystems : they are less than half those for southern taiga forest on podzolic soil and only one-tenth of that in mixed lowland forest (Table 5.4.5). The production estimate obtained in this study (360 g m^{-2} yr^{-1}) falls within a range of other values estimated for South African heathlands, although it was a little higher than values for a number of other mediterranean heathlands and shrublands. This is unexpected because South African systems are supposedly the poorest (Table 5.4.4). One possible reason might have been the duration of the experiment. The plots were harvested in June and many plant species may have undergone an important growth flush with the first rains of the season. This means that substantially more than 75 % of annual primary productivity had been realised by then and adjusting the productivity value by a full 25 % over-estimated the true value. Nevertheless, using this reasoning, an estimate of 270 g m^{-2} per annum would still fall in the upper part of the range for all mediteranean systems.

The results from the exclusion plot experiment suggested that herbivores consumed approximately 25 % of the available photosynthetic biomass over a period of 9 months during 1979 - 1980. Apart from antelope, hares Lepus capensis and porcupines Hystrix sp. were occasionally seen. Most evidence of herbivory however, was confined to 20 cm or more above ground (personal observation) and therefore was assumed to be antelope herbivory. The large variance in the biomass measurements both inside and outside the exclusion plots reflected the heterogeneous physiognomic structure of this vegetation. A few of the random quadrats were located in the very densest bush clumps while most were in more sparse areas. This obviously produced a skewed frequency distribution where the mean was much greater than the median. The difference between inside and outside woody biomass was fairly large since, as mentioned above, the

vegetation has low productivity (Table 5.4.4). This difference may also be due to vegetation heterogeneity. Similar magnitudes of differences in green and dry plant biomass were found in an exclusion plot experiment conducted in a grassland and monitored for about 12 years in Serengeti (McNaughton 1979): the differences in green biomass from inside to outside were small (64.4 and 58.8 g m^{-2} respectively), while differences in dry material were enormous (345.1 and 19.8 g m^{-2} respectively).

Since the productivity of the vegetation was so low, the 160 g m^{-2} dry weight of phytomass (calculated from Table 5.4.1) consumed by antelope represents fully one third of the production. This is higher than the 13 to 20% of plant biomass consumed by grazers in typical terrestrial grassland ecosystems. In forest ecosystems only 7.7% of production is consumed (Petrusewicz and Grodzinski 1975). Crawley (1983) provides 13 estimates which range from 1.0 - 10.6 % for trees and shrubs. Eight of the 13 consumers in this list were invertebrates. The estimate of consumption in this study was made on perennial plants and the time period that the exclusion plots were set up (October until June) meant that a large number of short-lived spring annuals were not included. However, these annuals, particularly the flowers, were eaten by the antelope (personal observation).

Loss of photosynthetic tissue can cause widely different short-term changes in resource allocation and production and it is difficult to predict the particular impact on plant fitness of a given level of herbivory. For example, Colorado potato beetles can consume 20% of the leaves of potato plants and the yield decreases by approximately 1% at this level of herbivory, while lepidopteran larvae can cause wood increment losses many times greater than the mass of plant material they consume (Varley and Gradwell 1962, Morrow and LaMarche 1978, Kulman 1971). The effects of plant defoliation on plant

fitness are extremely diverse and have been thoroughly reviewed by Krischik and Denno (1983).

The results of this study show that small antelope consume measurable amounts of biomass from the shrubland vegetation which they inhabit. This occurs in vegetation with low productivity and low biomass, both of which are characteristic of mediterranean-type ecosystems. These results provide a baseline should a more extensive production and biomass study of the vegetation be undertaken.

Table 5.4.1. Mean biomass (g m^{-2} d.w.) inside (n = 88) and outside (n = 88) four mediterranean shrubland exclusion plots after nine months. (Also see addendum on back cover).

		$\bar{x}$	± S.E.	t	p
Phytomass	inside	470	± 52		
				1.92	< 0.05
	outside	350	± 34		
Wood	inside	970	± 124		
				2.89	< 0.005
	outside	530	± 88		
Total biomass	inside	1440	± 166		
				2.81	< 0.005
	outside	880	± 110		

Table 5.4.2. Limits for the t values reported in Table 5.4.1, for the comparison of above ground biomass categories from the exclusion plot results. See text for details.

	Limits*		
Phytomass inside/outside	0.2318	-	- 0.0017
Wood inside/ outside	0.2980	-	0.1419
Total biomass inside/outside	0.9503	-	0.1697

* Calculated from: $\bar{x}_1 - \bar{x}_2 \pm \sqrt{SE_1^2 + SE_2^2} \cdot 1.96$

Table 5.4.3. Above ground plant biomass in some mediterranean-type and other low soil nutrient ecosystems.

Vegetation type	Location	Age (yrs)	Biomass[a] ($g\ m^{-2}$)	Reference
Fynbos	South Africa	22	950	Rundel 1983
Strandveld	South Africa	[b]	1440	Table 5.4.1, this study
Mature broad leaf scrub fynbos	South Africa	6 10	1570 1840	Kruger 1977 Kruger 1977
Renosterveld (Elytropappus rhinocerotis)	South Africa	[b]	1100	Rutherford 1978
Evergreen heath (Protea, Restionaceae)	South Africa	3 16	660 1240	Kruger 1977 Kruger 1977
Mature chaparral	California	[b]	2300	Mooney et al.1977
Matorral	Chile	14	740	Mooney et al.1977
Garrigue	France	17	2350	Rapp & Lossaint 1981
Sand heath Heath	Australia	18 15 25	1600 1850 2720	Jones et al. 1969 Specht et al. 1958 Specht 1969
Woodland (Eucalyptus socialis)	Australia	55	4020	Burrows 1976
Temperate deciduous forest	U.S.A. (Hubbard Brook)	55	6050	Whittaker et al. 1979

[a] $g\ m^{-2}$ is numerically equivalent to tonnes km^{-2}.

[b] date of last burning unknown.

Table 5.4.4. Primary production (g m^{-2}) of some mediterranean-type ecosystems. Some figures for South African grassland savanna is included for comparison.

Vegetation type	Production	Source
Strandveld	360	This study
Phrygana	202	Margaris 1975 in Mooney 1981
Montane fynbos	100-400	Kruger 1977
Chaparral	130	Mooney 1981
Garrigue	110	Lossaint 1973
Australian heathland	115	Kruger 1977
South African grassland		
(rainfall 600 mm/y)	120	Meredith et al. 1955
(" 700 ")	154	Kruger & Smit 1973 in Rutherford 1978
(" 800 ")	212	Rethman et al. 1971
Savanna & woodland		
(herbaceous layer)		
(rainfall 425 mm/y)	23-62	Le Roux 1972 in Rutherford 1978
(" 500 ")	100	"
(leaf and twig from woody layer)		
(rainfall 500 mm/y)	150	"

Table 5.4.5. Above ground plant biomass and productivity of five major ecosystem complexes on medium and high nutrient soils (Adapted from Olson 1975).

Ecosystem	Plant biomass ($g\ m^{-2}$ d.w.)	Productivity ($g\ m^{-2}\ y^{-1}$ d.w.)
Sub-polar mountain tundra	700	70
Southern taiga forest on podzolic soil	30 000	750
Cool northern broad-leaved forest	37 000	800
Warm evergreen and deciduous lowland forest on red and yellow soils	45 000	2 000
Montane semi-arid woodlands on brown soils	12 000	1 300

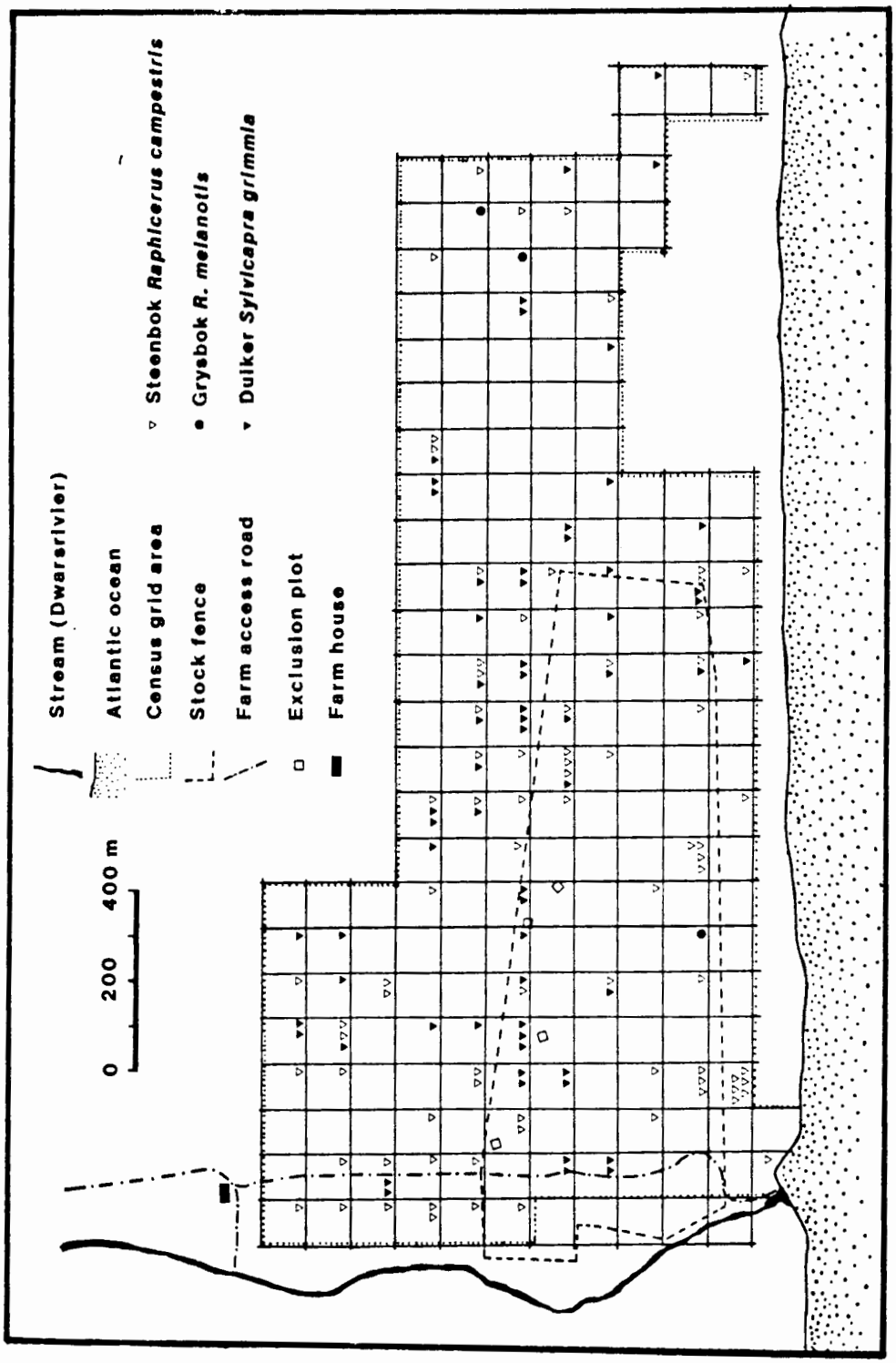


Figure 5.4.1 The location of the study site on Rondeberg farm, indicating the location of the four exclusion plots inside the stockfenced area and the distribution and abundance of antelope sightings in 1 ha blocks from February 1979 - May 1980.

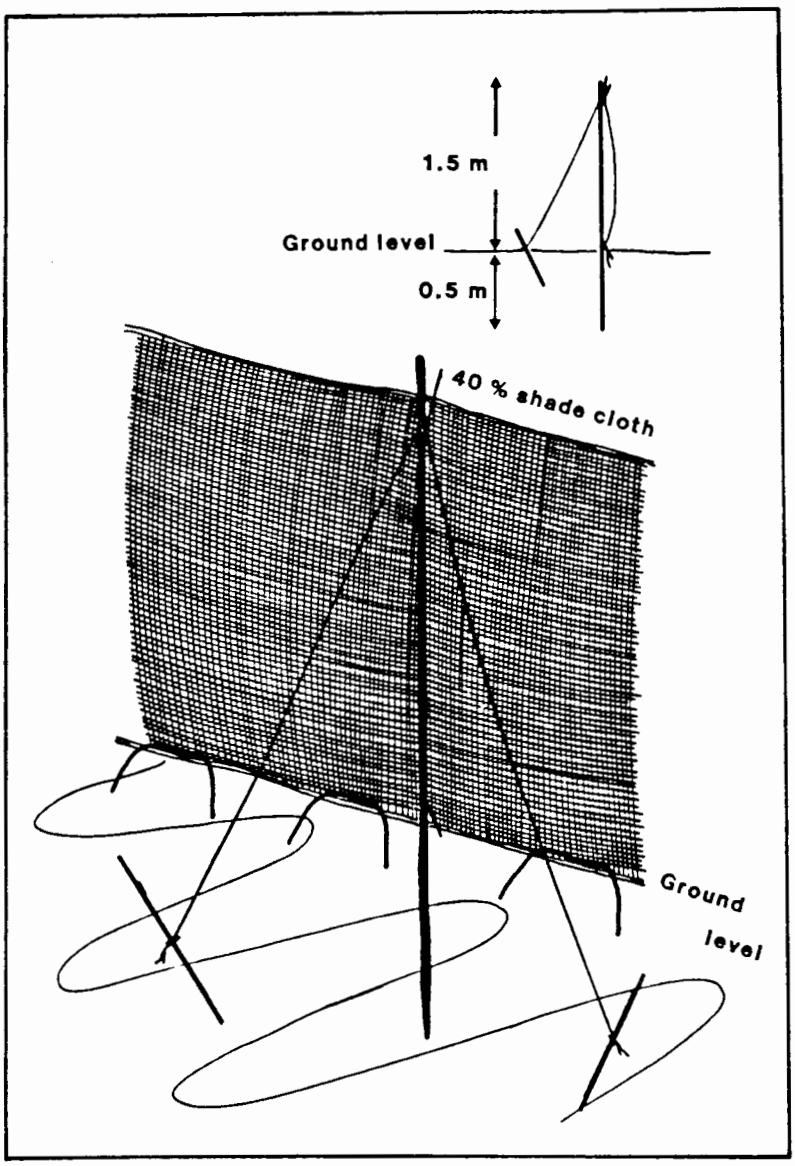


Figure 5.4.2 Diagram showing exclusion plot construction.

6.0 French garrigue and maquis

The Mediterranean basin covers the largest land area (60 %) of all the mediterranean ecosystems on the planet (Di Castri 1981, Quezel 1981) and contains approximately 410 000 km^2 of natural vegetation (Le Houerou 1981). The largest contiguous vegetation formations occur in Spain, Turkey, Morocco and Italy (di Castri 1981) and much of this is not climax vegetation (Tomaselli 1981b, Pons 1981). There is a complex interrelationship between physiognomy and succession which has led to some confusion about terms, exacerbated by many local language differences. Tomaselli (1981) proposed using matorral and high, middle or low to describe successional stage, with dense, discontinuous and scattered to describe physiognomy. Maquis is the French name for high matorral, while garrigue is middle matorral, found on calcareous substrates. The name garrigue is derived from a common name for Quercus coccifera, the most abundant species in the community (Tomaselli 1981). In older ecological literature, any differences in successional type were almost always described floristically (Tomaselli 1981) but in recent years French ecologists have begun to distinguish maquis growing on siliceous from garrigue growing on calcareous substrates (Bottner 1970, Bottner & Boukris 1969, Specht 1981). Maquis and garrigue and their equivalent types cover approximately 250 000 km^2 or 60 % of all mediterranean ecosystems (Le Houerou 1981). Quezel (1981) provides a region-by-region description and a map of this distribution.

The species composition of these ecosystems is variably diverse and very complex. Three continents surround the mediterranean basin and all have contributed plant species (Pons 1981), while human habitation for 8 000 years has certainly had far-reaching effects on plant community composition (Le Houerou 1981). Approximately 29 plant

families, with at least 75 genera and hundreds of species occur in matorral shrublands. The Fabaceae, Lamiaceae and Cistaceae contain 15, 10 and 5 genera respectively, while other families are mono-generic. Some genera occur uniformly throughout the basin e.g. Artemisia, Jasminum, Juniperus, Myrtus, Olea, Phillyrea, Pinus, Pistacia, Ruscus and Ziziphus. Others occur in the western mediterranean e.g. Genista, Cistus, Cytisus, Thymus, Erica and Asparagus, and the eastern mediterranean e.g. Hypericum, Salvia, Satureja and Rhamnus. Quercus and Linum are poorly represented in the central basin but show increasing species numbers east and westwards (Quezel 1981).

This study comprising a single chapter examines differences in the chemical composition of Quercus coccifera which had been subjected to different edaphic and biotic regimes in a field experimental situation. Manipulation of soil nutrient conditions by artificial fertilization provides a method for examining the importance of soil nutrients in mediating the interactions between plants and herbivores. The close physical proximity of garrigue and maquis, with many species in common but growing on different substrates, provides a natural control for a fertilization experiment.

The research described in the next three chapters is devoted to studies of oaks in France and California. The mediterranean basin and California have strong affinities with each other (Di Castri 1981). The physiognomic structure of garrigue, maquis and chaparral is very similar and oaks Quercus spp. are important in both these ecosystems. The highest levels of soil nitrogen of all mediterranean ecosystems (0.2 - 0.5 %) occur in the mediterranean basin (Figure 4.0.1) while phosphorus levels here are intermediate (0.02 - 0.06 %) and are similar to those in California.

6.1 TANNIN POLYPHENOLS, NUTRITIONAL COMPONENTS AND INSECT HERBIVORY ON SOME GARRIGUE AND MAQUIS PLANT SPECIES

6.1 INTRODUCTION

Comparisons of mediterranean climate zones have mostly comprised vegetation and soil studies (Di Castri & Mooney 1973, Cody & Mooney 1978, Milewski 1983) with less attention being given to animals, except for birds (Cody 1973, Cody & Mooney 1978, Blondel 1984). Very little information has been published about plant-animal interactions (see Section 4.0), particularly herbivory.

In the previous section (Chapters 5.1 to 5.4), evidence was presented that antelope browsing South African shrubland plant species encountered a wide range of tannin polyphenols in the leaf tissue. The maximum levels in a few species were exceptionally high and average levels were in the range reported for nutrient poor ecosystems such as tropical forest. It is therefore important to determine what actual role substrate fertility plays in this and what effect browsing, combined with edaphic factors, has on the levels of tannin polyphenols in plants. A satisfactory field experiment with manipulation of both variables would certainly have taken years to develop, given the characteristically slow growth of plant species in mediterranean systems. However, lower soil fertility is an important feature of most mediterranean ecosystems. Therefore examination of some artificial or natural differences in soil fertility at a suitable site should reveal some interesting information about edaphic factors, carbon gain and allocation(primary production). These, in turn, would affect primary consumption and consumers. An area of garrigue vegetation near Montpellier, southern France, which has been the site of long term studies of mineral cycling (Rapp & Lossaint 1981), burning (Trabaud 1981, Lepart & Trabaud 1980) and stock utilization (Daget & Poissonet 1972, Thiault et al. 1979, Poissonet et al. 1982), provided an excellent study site for a preliminary study of herbivory.

In this study, I wished to see, firstly, whether differences between garrigue and maquis plant species were manifest at the primary consumer level and, secondly, to determine whether soil fertility affected nutritional and chemical qualities of the plants and whether this in turn affected primary consumption. I was interested in examining the interaction of insect herbivory, livestock browsing, soil fertilization and tannin polyphenols in garrigue and maquis communities. Specifically, I asked the following questions: 1) Do garrigue plant species incur greater levels of insect herbivore damage than maquis species (because garrigue is reputedly more fertile than maquis (Bottner 1970)); 2) Are there changes in tannin polyphenols which reflect the insect damage patterns; 3) In garrigue, what effect does burn frequency have on the patterns of insect damage and also on levels of tannin polyphenols; 4) Does heavy stock utilization affect the levels of insect damage and what effect does combined insect damage and stock utilization have on tannin polyphenols; 5) What effect does soil fertilization have on insect damage and on the levels of tannin polyphenols; and 6) How does stock browsing combined with soil fertilization affect tannin polyphenol levels?

6.1 METHODS

Study sites

This study was undertaken at three localities in southern France (Figure 6.1.1). The two sites close to Montpellier (Commune d'Aumelas 43°37'N, 3°38'E and Puech-du-Mas-du-Juge at St-Gely-du-Fesc 43°40'N, 3°51'E) were both situated in garrigue vegetation (Dugrand 1964). The third site at Col du Canadel (43°10'N, 6°32'E) was located on a fairly steep sea-facing slope in maquis vegetation. The

distinction between these two similar types of vegetation is based on associated soil types rather than on plant species composition (described in Section 6.0); garrigue occurs on a calcareous substrate and maquis on a siliceous material (Bottner 1970). Aumelas has an uncertain history of land use (Trabaud, personal communication) but appeared to be heavily browsed by stock animals (personal observation). The site at St. Gely has been used for a major fire experiment (Trabaud 1979, 1981, 1983), and an applied study on stock utilization of garrigue (Thiault et al. 1979, Poissonet et al. 1982).

Experimental design and plant collection

Collection and extraction of plant material was carried out as described in section 5.2. Approximately 500 g of leaf material was collected from each plant species at each of the three sites during December 1980 to February 1981. Collecting was done from as many individual plants of each species as possible. Care was taken not to damage leaves and to work quickly so as to minimise bias in collecting. All samples were subdivided into three portions and each portion was treated as follows. One portion of fresh leaf material (3 g) was homogenised under 100 % methanol using a Tissumizer ®. Each of the methanol samples was then sealed; further extraction and analysis was completed approximately three months later at Stanford University. A subsample of approximately 100 randomly picked leaves was taken from the remaining leaves. These were fixed with quick-drying glue to sheets of paper, pressed for 48 hours and photocopied. Each page was copied twice. The first copy gave an estimate of insect damage and on the second, the outline of each leaf was filled in as accurately as possible as if no insect damage had occurred, to give an estimate of undamaged leaf area. The paper leaf copies were then cut out and weighed with an accuracy of one hundredth of a milligram. Total leaf areas were calculated by proportion based on the weight of a

known area of paper. The difference between the two sets of leaves gave the leaf area eaten. This method was found to be accurate to 0.5% of the total leaf area. All the remaining leaves were weighed, oven-dried to constant mass at 55°C and weighed again to give wet : dry weight ratios. The leaves were stored in paper bags inside plastic bags, and later approximately 100 g of this material was ground in a Wiley mill through 20 mesh for nitrogen and phosphorous determination (methods described in Chapter 7.1).

Insect damage was assessed in relation to four abiotic and biotic factors: occurrence of plant species on different soil types, stock utilization combined with soil fertilization, and different burn frequencies.

Vegetation on a siliceous substrate (maquis) which apparently had not been burnt for at least the previous decade (Schvester, personal communication) was located 120 km east of Montpellier at Col du Canadel. Leaf material was collected there from the same species occurring at St Gely du Fesc, permitting comparison between the two vegetation subtypes. Stock browsing and fertilizer effects were studied at Aumelas and St Gely du Fesc. Aumelas had no stock fencing and had obviously been heavily browsed. St Gely, the site of fire and pasture research, was carefully fenced and the history of different patches of vegetation was documented (Trabaud 1979, 1983, Poissonet et al. 1982, Godron et al. 1981, Thiault et al. 1979). Comparison of Aumelas and an area inaccessible to stock at St Gely provided data for browsing effects. The stocking intensity where the plant material was collected was 3 sheep per hectare from 1970 -1976 and 5 sheep per hectare from 1976 - 1980. An area at St Gely which had had controlled fertilizer application of 200 kg per hectare per year provided a comparison of fertilization effects. The combined effects of browsing and fertilization were studied in an area

which had been stocked at the above-mentioned rate and fertilized with 300 kg per hectare per year. Finally, the burn plots adjacent to the pasture research project provided vegetation subject to different fire regimes for assessing the effects of fire on levels of insect damage.

6.1 RESULTS

Comparison of garrigue and maquis plant species

A pair-wise comparison (pair-wise t-test) of foliar N and P levels in all five species growing on both substrate types showed that N levels were significantly higher in garrigue species (one-tailed $t = 2.62$, $df = 4$, $p < 0.050$) while P levels were significantly higher in maquis (one-tailed $t = -2.27$, $df = 4$, $p < 0.050$) (Appendix 6.1.1). Leaf water content was also significantly higher in maquis (one-tailed $t = -2.59$, $df = 4$, $p < 0.050$) (Appendix 6.1.1).

In almost all chemical tests (24 / 30) on all five plant species, tannin polyphenol levels were higher in maquis species (Table 6.1.1). These differences were significant for both total polyphenols and astringency in Quercus coccifera and Cistus monspeliensis and not significant for Phillyrea angustifolia. Quercus ilex and Pistacia lentiscus had insufficient sample sizes for statistical comparison. In Q. ilex, total polyphenols were approximately 10 % higher while the astringency was 40 % higher in the maquis sample. In P. lentiscus, total polyphenols were 35 % higher, condensed tannins were almost three times higher and, anomalously, astringency was 50 % lower in the maquis sample. Condensed tannins in the other four species showed small differences which were not

significant between maquis and garrigue plants.

Total leaf area of the five species occurring on both substrate types was significantly greater in four of the five maquis species (Table 6.1.2). The absolute area of insect damage per leaf was significantly greater in three of the five maquis species. However, I compared the damage as a ratio of the total leaf area because of the range of leaf sizes at both study sites. By this transformation, damage was greater on P. angustifolia and C. monspeliensis in maquis, while P. lentiscus showed greater damage in garrigue. The two oak species showed no significant difference in area of damage. These data (Table 6.1.2) do not indicate any clear distinction between garrigue and maquis on the basis of evident insect herbivory.

Browsing and fertilization effects on garrigue

Heavily browsed Q. coccifera at Aumelas had astringency levels which were significantly elevated by approximately 2 % although total polyphenols and condensed tannins were not significantly different at either site (Table 6.1.3). The level of astringency in P. angustifolia which was heavily browsed, was significantly higher by approximately 1%, while total polyphenols were approximately 0.5% higher. There were no significant differences in Q. ilex, although levels of all measured tannin polyphenols were slightly higher in plants from the heavily browsed site. Levels of both N and P were higher in heavily browsed oaks (Appendix 6.1.1).

Total leaf area was significantly smaller in heavily browsed Q. coccifera growing at Aumelas and significantly larger in Q. ilex (Table 6.1.4). Absolute areas of insect damage per leaf were not significantly different in either oak species at either site.

However, with transformation of damage to a ratio of total leaf area, heavily browsed Q. coccifera at Aumelas had significantly more insect damage. Q. ilex showed no significant difference in ratios of insect damage at either sites.

Foliar N levels at fertilized sites were significantly elevated (pair-wise one-tailed $t = 3.11$, $df = 2$, $p < 0.050$) in a comparison of means for three species (Table 6.1.5). Foliar P levels were slightly higher but not significantly so. Whole leaf areas were significantly larger in Q. coccifera and Q. ilex which had been fertilized (Tables 6.1.6a and 6.1.6b). Absolute areas of damage were also significantly larger as were ratios of damage to total leaf area. The interactive effects of browsing and fertilizing on tannin polyphenol levels on Q. coccifera are summarised in Table 6.1.7 and the data tabulated in Appendix 6.1.2. Browsing appeared to raise the levels of condensed tannins while, conversely, fertilization tended to depress them irrespective of whether browsing had occurred or not. The lowest levels of condensed tannins occurred in Q. coccifera plants which were both browsed and fertilized. Only the condensed tannins were significantly affected by simultaneous browsing and fertilization. Total polyphenols and astringency followed some of the same trends but these were not statistically significant. Foliar N and P responded similarly in that the lowest levels of N and P were found in unbrowsed and unfertilized plants, while combined browsing and fertilization appeared to produce the highest foliar nutrient levels. These responses could not be tested because of the small sample sizes for N and P levels.

The effect of burn frequency

The tannin polyphenol composition of Q. coccifera was significantly altered by increased frequency of burning (Table 6.1.8). Both total polyphenols and astringency in the pure

methanol extract showed significant increases. Total polyphenols increased by 1.5 % when comparing unburnt Q. coccifera with Q. coccifera which had been subjected to a burn frequency of 6 years; total polyphenols declined slightly when comparing the 6-year burn frequency with the 2-year frequency. These small differences in chemical concentrations, although statistically significant, were based on small sample sizes and this suggests that their biological significance may be small.

Increased burning frequency decreased the leaf size of Q. coccifera (Table 6.1.9) and increased the ratio of insect damage per leaf. There was no significant change in mean leaf area when comparing unburnt plants with those burnt on a 6-year frequency, but there was a significant increase in insect damage by approximately 6 %. Conversely, there was a significant decrease by approximately 20 % in leaf size in plants subjected to a 6-year burn frequency compared with a 2-year burn frequency. There was, however, no significant increase in insect damage.

6.1 DISCUSSION

Experimental approaches to ecological studies can reveal very important and often masked facets of ecosystem dynamics, even if caution is advised in generalising from such findings. If the process in question is subtle or protracted, this usually makes experiments or perturbations impractical or logistically difficult. This problem was greatly diminished by taking advantage of the St. Gely du Fesc site. This experimental study focused on leaf size, leaf chemical content and levels of insect herbivory on five plant species growing in mediterranean vegetation defined as French middle matorral (Tomaselli 1981). The findings are divided into four categories, three of which are mainly concerned with Q. coccifera and Q. ilex on calcareous soils ('garrigue" sensu stricto), while the fourth discusses differences in the same five species on calcareous(garrigue) and on siliceous soils (maquis). This last category is discussed first.

The leaves of the maquis species contained significantly higher levels of Phosphorous (P) and water, and they were approximately 25 % bigger in area than garrigue leaves; garrigue leaves contained significantly higher levels of Nitrogen (N). There were insufficient replicates in some species to compare all five species for polyphenol levels. However, both Q. coccifera and C. monspeliensis in maquis contained significantly higher levels of total polyphenols and were more astringent, but there was no significant difference in levels of condensed tannins. There was also no significant difference in total polyphenols and astringency in P. angustifolia while condensed tannins could not be compared. Insufficient sample sizes of Q. ilex and P. lentiscus prevented testing for significant differences in those species. Nevertheless, for both species, individuals growing in maquis appeared to have higher levels of polyphenols. These differences,

however, did not translate into any clear-cut pattern in levels of insect damage. Two species in maquis showed significantly higher insect damage than their counterparts growing in garrigue, while the other two species had significantly more damage growing in garrigue. The fifth species, Q. coccifera, had slightly more damage in garrigue but this was not statistically significant. These results indicate that natural differences in the levels of N in the soils were directly reflected in differences in the leaf tissue: garrigue species grew in higher substrate N conditions and had higher foliar N levels. The lower levels of N in the substrate in maquis, together with the higher levels of polyphenols in species growing in maquis, suggest that the plants at this site were accumulating and storing carbon in this form. However, despite these differences, no clearly discernible pattern of insect damage emerged from these results, which suggests that maquis and garrigue species represent a very similar food source for insects.

Heavy stock browsing introduced some apparently contradictory changes in these plants. Q. coccifera, which was heavily browsed, had leaves which were significantly more astringent than those of unbrowsed plants. While total polyphenols and condensed tannins were higher in the browsed plants, the differences were not significant. Both total polyphenols and astringency in P. angustifolia were significantly higher. However, in Q. ilex there were no significant differences in the levels of polyphenols. There is some observational and experimental evidence that plant defense can be induced by both vertebrate and invertebrate herbivore damage (e.g. Green & Ryan 1972, Haukioja & Niemela 1978, Haukioja 1980, Bryant 1981). This evidence has been well reviewed by Rhoades (1979, 1983). Leaves of heavily browsed Q. coccifera were significantly smaller by approximately 20 % in area than plants from the unbrowsed site. The opposite was found in Q. ilex: leaves of plants occurring at the heavily browsed site were significantly larger by 20 %. However, foliar N and P levels were significantly

higher in all three of these heavily browsed species and furthermore there was significantly more insect damage on Q. coccifera. One explanation is that the very heavy stock usage simultaneously introduced some degree of fertilization with manure. This effect was found in Serengeti, where growth stimulation in grasses was caused by nutrients recycled from dung and urine from animals (McNaughton 1979). This would raise the observed foliar N values which would improve these plants as a food resource for insect herbivores (e.g. Van Emden 1966).

This explanation does not account for the inefficacy of raised polyphenol levels in defending the plants at the heavily browsed site, if it is assumed that increased polyphenol levels were induced by heavy browse pressure in the first place. It is possible, however, that site differences between St Gely (garrigue, not browsed) and Aumelas (garrigue, heavily browsed), although spatially close and apparently very similar, may account for the differences in leaf chemistry. Artificial soil fertilization significantly increased the levels of foliar N in Q. coccifera, Q. ilex and P. lentiscus, but P levels were unchanged. The mean leaf area of Q. coccifera was almost doubled while Q. ilex leaf area increased by at least 50 %. Also, a significant increase in the area of insect damage in both oak species correlated with artificial soil fertilization. There is a diversity of evidence which shows that substrate nutrient levels directly affect the biosynthesis of polyphenols. This has been shown in tissue culture experiments (Westcott & Henshaw 1976), whole plant experiments (Del Moral 1972), by comparisons of several plant species growing under different natural soil fertility levels (Davies et al. 1964, McKey et al. 1978) and by field fertilization experiments with a single plant species, Lotus pedunculatus (Barry & Forss 1983). Condensed tannin concentrations in L. pedunculatus decreased from 8 - 11 % d.w. to 2 - 3 % d.w. when fertilizer (P and sulphur(S)) was applied to low fertility acid soil. All these studies

provide evidence that polyphenol levels, in particular, increase in plant tissue when the substrate nutrient levels, specifically N, decline. In addition, the concentration of numerous other compounds, including cyanogenic glycosides, glucosinolates, alkaloids and terpenoids, is affected by nutrient and water stress (Gershenzon 1984).

The interaction of browsing and artificial fertilization in affecting leaf chemistry was complex. Based on existing theory, I would have expected that browse as a form of stress would tend to raise polyphenol levels (Rhoades 1979, 1983, Chapin 1980b) while soil fertilization would tend to decrease them (Del Moral 1972, Barry & Forss 1983) and this antagonism would tend to minimise any measurable chemical effects. These 'hypotheses' are supported to some extent by the data. Soil fertilization raised foliar levels of N and P in Q. coccifera whether or not it was browsed. However, there was no significant interactive effect for browsing combined with fertilization. The responses of polyphenol levels was surprising: there were no significant interactive effects for total polyphenols or astringency in Q. coccifera but condensed tannins increased if the plant was browsed but unfertilized. They decreased a little if the plant was fertilized, and if the plant was fertilized and browsed they decreased by 50 %. Similar increases and decreases occurred in the other two measures of polyphenols individually but the interactive effects were insignificant (Appendix 6.1.2). Unfortunately, data were not available to examine whether changes in condensed tannins had any effect on leaf area and insect damage levels.

Finally, increased frequency of burning decreased foliar levels of N and P slightly in Q. coccifera, while both total polyphenols and astringency increased. Condensed tannins did not change significantly. Leaf size was not significantly different in plants burned with 6-year frequency compared with unburnt plants. More frequent burns (at 2-year

intervals) decreased leaf size by 20%. Insect damage was significantly higher on plants from burnt sites even if they were burnt at the lowest frequency of 6-year intervals. It appears that the changes in total polyphenols and astringency might have been effective in controlling the level of damage, since there was no further increase in levels of insect damage from a 6-year frequency to a 2-year frequency. A study of the soil chemical changes from 1969 - 1974 at this study site (Trabaud 1983) suggested that soil N decreased with increased fire frequency and this, in turn, increased the C : N ratio. Soil N decreased from 0.4 % to 0.3 % and this translated into an increase in the C : N ratio from 14 to 17. Although relatively small, this decrease in the level of soil N may be a partial explanation for the increased polyphenol levels in the more frequently burnt Q. coccifera .

The levels of foliar N, as measured in this study, are consistent with the relative levels of N in the soil. The N in garrigue species (1.44 ± 0.10 %) converts into approximately 9 % protein using a conversion factor of 6.25 (Robbins 1983), while that for maquis species (1.29 ± 0.16 %) converts into 8 % protein. In terms of seasonal changes which might occur, these differences are probably biologically insignificant. In mediterranean-type ecosystems, the mediterranean basin has soils containing the highest levels of soil N (0.2 - 0.5 %)(Figure 4.0.1). In California where the soil contains much lower levels of N but similar levels of P (Figure 4.0.1), in Heteromeles arbutifolia , protein declines from 13.1 % in spring (new leaves) to approximately 6.2 % by midwinter (Dement & Mooney 1974). Protein in South African strandveld species is lower than this, varying between 5.9 and 7.9 % (Section 5.4), reflecting the very low levels of soil N found in this ecosystem. Compared with tropical vegetation, also found on low nutrient substrates, these values are low: e.g. afro-alpine vegetation has a mean level of 15.5 % protein and rain forest 13.8 % (Waterman et al. 1983). In neo-tropical

lowland rain forest, mature pioneer plants have foliar protein levels ranging from 10.6 - 19.4 %, while mature persistents contain 7.5 - 19.4 % (Coley 1983).

As a short term piece of research, with many practical and logistical constraints, this study nevertheless proved to be useful. The findings indicate that low nutrient mediterranean shrublands respond to artificial and natural fertilization in physiologically similar and predictable ways: increased fertilization leads to increased foliar N levels and decreased polyphenol concentrations. Natural substrate differences in nutrients translated into the same biochemical changes in the plants as artificial fertilization. Heavy stock browsing correlated with increases in the levels on polyphenols in the browsed plant species. However, no clear pattern emerged about insect utilization patterns, based on evident leaf damage. It is possible that a seasonal study would show a clearer pattern, since fluctuations in levels of defoliation could be measured simultaneously with measurements of leaf chemistry.

Table 6.1.1. Tannin polyphenol levels (mg/g d.w.) in 100% methanol (A)extracts, and 100% + 50% methanol (A+B) extracts in five plant species occurring both in garrigue and maquis. (mg/g x 0.1 = %)

		Garrigue		Maquis		t	p
		$\bar{x}$	S.D.	$\bar{x}$	S.D.		
Quercus coccifera		(n = 7)		(n = 4)			
Tot. polyphenols	A	72.9	11.6	91.2	7.7	3.14	< 0.02
	A+B	96.5	12.9	111.3	4.5	2.76	< 0.05
Cond. tannins	A	39.5	18.5	43.7	4.6	0.57	N.S.
	A+B	48.3	17.2	50.8	7.9	0.33	N.S.
Astringency	A	83.2	13.8	150.2	38.3	3.38	< 0.01
	A+B	106.6	15.0	174.2	38.8	3.34	< 0.01
Quercus ilex		(n = 2)		(n = 1)			
Tot. polyphenols	A	67.2	11.0	71.7	-		
	A+B	81.4	11.0	89.8	-		
Cond. tannins	A	88.7	57.2	73.2	-		
	A+B	106.2	65.6	86.9	-		
Astringency	A	92.4	9.2	130.3	-		
	A+B	111.3	10.5	151.6	-		
Pistacia lentiscus		(n = 3)		(n = 1)			
Tot. polyphenols	A	120.9	12.0	162.2	-		
	A+B	145.3	18.3	195.8	-		
Cond. tannins	A	188.8	42.8	540.9	-		
	A+B	234.9	73.8	629.9	-		
Astringency	A	117.6	47.8	54.0	-		
	A+B	145.7	46.9	97.5	-		

cont.

Table 6.1.1 (cont.)

Phillyrea angustifolia		(n = 2)		(n = 2)			
Tot. polyphenols	A	66.5	5.4	70.5	12.9	0.40	N.S.
	A+B	70.3	5.2	75.2	14.1	0.46	N.S.
Cond. tannins	A	62.5	67.4	11.5	-*		
	A+B	62.5	67.4	11.5	-		
Astringency	A	52.2	13.2	76.2	10.7	2.0	N.S.
	A+B	61.7	18.1	83.1	20.5	1.11	N.S.
Cistus monspeliensis		(n = 3)		(n = 3)			
Tot. polyphenols	A	41.1	5.3	60.7	7.5	3.70	< 0.05
	A+B	60.2	3.9	82.9	9.2	3.93	< 0.02
Cond. tannins	A	52.2	24.8	92.7	43.4	1.40	N.S.
	A+B	74.4	40.7	128.6	56.1	1.35	N.S.
Astringency	A	73.1	8.2	102.7	8.8	4.28	< 0.02
	A+B	119.8	6.4	140.2	7.3	3.64	< 0.05

*one sample was lost before chemical analysis was completed

Table 6.1.2. Comparison of a) leaf size (mm^2) and b) relative amount of damage per leaf(% arcsin transformed) occurring on five plant species in maquis and garrigue shrub vegetation in southern France.

a) Total leaf area

	Garrigue			Maquis			t	p
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n		
Quercus coccifera	169.61	85.85	135	211.90	106.81	136	3.59	< 0.001
Q. ilex	261.98	112.46	133	334.69	142.07	135	4.65	< 0.001
Pistacia lentiscus	72.33	32.79	115	74.16	30.24	145	0.46	N.S.
Phillyrea angustifolia	115.07	50.65	270	168.78	78.10	143	7.44	< 0.001
Cistus monspeliensis	78.80	25.66	95	131.81	77.14	75	5.71	< 0.001

b) % damage (arcsin transformed)

	Garrigue			Maquis			t	p
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n		
Q. coccifera	19.12	9.57	125	18.74	8.75	110	0.32	N.S.
Q. ilex	18.94	10.19	125	20.96	10.89	130	1.53	N.S.
P. lentiscus	24.60	9.61	108	20.57	10.50	129	3.08	< 0.0025
P. angustifolia	17.74	8.13	212	21.42	11.37	127	3.20	< 0.001
C. monspeliensis	17.64	8.32	86	22.64	12.94	68	2.77	< 0.005

Table 6.1.3. Comparison of polyphenols(mg/g d.w.) in 100% methanol (A) extracts and 100% + 50% methanol (A+B) extracts of leaves of two oak species occurring in an area of garrigue heavily browsed by stock (Aumelas) and in unbrowsed garrigue (St. Gely). (mg/g x 0.1 = %)

		Unbrowsed			Heavily browsed			t	p
		$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n		
Quercus coccifera									
Tot. polyphenols	A	72.89	11.60	7	79.86	17.27	3	0.64	N.S.
	A+B	96.49	12.86	7	100.80	23.36	3	0.30	N.S.
Cond. tannins	A	39.49	18.54	7	48.32	20.18	3	0.65	N.S.
	A+B	48.32	17.21	7	58.26	23.06	3	0.67	N.S.
Astringency	A	83.19	13.83	7	105.60	2.99	3	4.07	< 0.001
	A+B	106.55	14.96	7	125.20	10.35	3	2.27	< 0.025
Quercus ilex									
Tot. polyphenols	A	67.20	11.03	2	67.40	7.56	3	0.02	N.S.
	A+B	81.40	11.00	2	82.26	7.22	3	0.10	N.S.
Cond. tannins	A	88.71	57.20	2	80.87	26.55	3	0.17	N.S.
	A+B	106.19	65.57	2	94.27	38.28	3	0.23	N.S.
Astringency	A	92.41	9.21	2	92.72	20.60	3	0.02	N.S.
	A+B	111.25	10.51	2	121.69	23.09	3	0.68	N.S.
Phillyrea angustifolia									
Tot. polyphenols	A	66.50	5.37	2	71.30	5.47	3	0.97	N.S.
	A+B	70.30	5.23	2	75.60	4.91	3	1.14	N.S.
Cond. tannins	A	-	-	-	-	-	-		
	A+B	-	-	-	-	-	-		
Astringency	A	52.22	13.24	2	65.36	10.29	3	1.19	N.S.
	A+B	61.7	18.09	2	75.00	13.80	3	0.88	N.S.

Table 6.1.4. Comparison of a) leaf size (mm^2) and b) insect damage (% area arcsin transformed) in two oaks Quercus coccifera and Q. ilex at a garrigue site which was heavily browsed by stock (Aumelas) and a garrigue site which was unbrowsed (St Gely).

a) Total leaf area

	Unbrowsed			Heavily browsed			t	p
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n		
Quercus coccifera	169.61	85.85	135	133.24	56.34	132	4.10	< 0.0005
Q. ilex	261.98	112.46	130	310.26	92.23	135	3.84	< 0.0005

b)% damage (arcsin transformed)

	Ungrazed			Heavily grazed			t	p
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n		
Q. coccifera	19.12	9.57	121	23.02	9.77	125	3.17	< 0.001
Q. ilex	18.94	10.19	125	17.52	7.04	130	1.29	N.S.

Table 6.1.5. The effect of soil fertilization on the levels of a) foliar nitrogen (% d.w.) and b) phosphorus (d.w.) in three garrigue species.

a) Nitrogen (%)

	Fertilised			Unfertilised			df	t (pair-wise)	p
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n			
Q. coccifera	1.58	0.01	2	1.40	0.10	2			
Q. ilex	1.66	0.24	2	1.48	0.04	2	2	3.11	< 0.05
P. lentiscus	1.61	-	1	1.18	-	1			

b) Phosphorus

	Fertilised			Unfertilised			df	t	p
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n			
Q. coccifera	0.088	0.010	2	0.064	0.008	3			
Q. ilex	0.079	0.012	2	0.077	0.017	2	2	1.96	N.S.
P. lentiscus	0.113	-	1	0.071	0.021	2			

Table 6.1.6. Comparison of the effect of soil fertilization on a) leaf area (mm^2) and on b) the ratio of insect damage to total leaf area in two garrigue oak species.

a) Leaf area

	Fertilised			Unfertilised			t	p
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n		
Q. coccifera	319.92	149.19	117	169.61	85.85	135	9.60	< 0.0005
Q. ilex	399.28	161.35	120	261.98	112.46	133	7.80	< 0.0005

b) Damage : total leaf area (% arc sin transformed)

	Fertilised			Unfertilised			t	p
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n		
Q. coccifera	22.92	10.15	109	19.12	9.57	121	2.90	< 0.005
Q. ilex	21.88	12.26	76	18.94	10.19	125	2.94	< 0.005

Table 6.1.7 Results of a two way ANOVA (SAS-GLM procedure ANOVA SS type 3) on fertilization and browsing effects on the chemical composition of Quercus coccifera leaves. Data are provided in Appendix 6.1.2.

TOTAL POLYPHENOLS

		A. 100% MeOH extract			B. 50% MeOH extract			TOTAL (A+B)		
Effect	df	SS	F	p	SS	F	p	SS	F	p
Browse	1	15.34	0.22	>0.1	0.024	0.00	>0.1	129.85	0.71	>0.1
Fertilization	1	20.63	0.30	>0.1	4.84	0.74	>0.1	206.61	1.13	>0.1
Browse/Fertilization	1	343.19	4.91	<0.05	4.55	0.69	>0.1	562.91	3.08	>0.1
Error	15	1048.93			98.25			2739.21		

ASTRINGENCY

		A. 100% MeOH extract			B. 50% MeOH extract			TOTAL (A+B)		
Effect	df	SS	F	p	SS	F	p	SS	F	p
Browse	1	134.92	2.59	>0.1	28.53	3.62	<0.1	39.35	0.59	>0.1
Fertilization	1	51.17	0.98	>0.1	1.95	0.25	>0.1	73.10	1.09	>0.1
Browse/Fertilization	1	617.18	11.83	<0.005	3.44	0.44	>0.1	712.80	10.61	<0.01
Error	15	782.73			118.30			1007.27		

CONDENSED TANNIN

		A. 100% MeOH extract			B. 50% MeOH extract			TOTAL (A+B)		
Effect	df	ss	F	p	SS	F	p	SS	F	p
Browse	1	1.93	0.02	>0.1	1.67	0.13	0.1	0.10	0.00	>0.1
Fertilization	1	890.66	7.00	<0.025	22.05	1.78	0.1	1193.16	8.21	<0.025
Browse/Fertilization	1	173.22	1.36	>0.1	11.59	0.94	0.1	274.34	1.89	>0.1
Error	14	1781.26			173.41			2033.86		

Table 6.1.8. One way analysis of variance of effects of different burn frequencies (2 years, 6 years and control) on the chemical composition of Quercus coccifera (df 2,1).

Chemical test	F ratio	p
Total polyphenols		
A extract	4.91	< 0.05
A+B	3.83	N.S.
Condensed tannins		
A extract	0.24	N.S.
A+B	0.90	N.S.
Astringency		
A extract	6.31	< 0.05
A+B	6.90	< 0.05

Table 6.1.9. Effect of different burn frequencies on leaf area (mm^2) of and insect damage (% leaf area damaged, arcsin transformed) to Quercus coccifera.

a) Total leaf area

	$\bar{x}$	S.D.	n	t	p
Unburnt	184.38	87.55	142	0.99	N.S.
6 year burn frequency	174.01	82.04	120		
2 year burn frequency	138.01	52.14	100	3.94	< 0.001

b) % damage

	$\bar{x}$	S.D.	n	t	p
Unburnt	18.95	8.90	261	4.30	< 0.001
6 year burn frequency	24.30	11.81	112		
2year burn frequency	24.24	11.81	97	0.04	N.S.

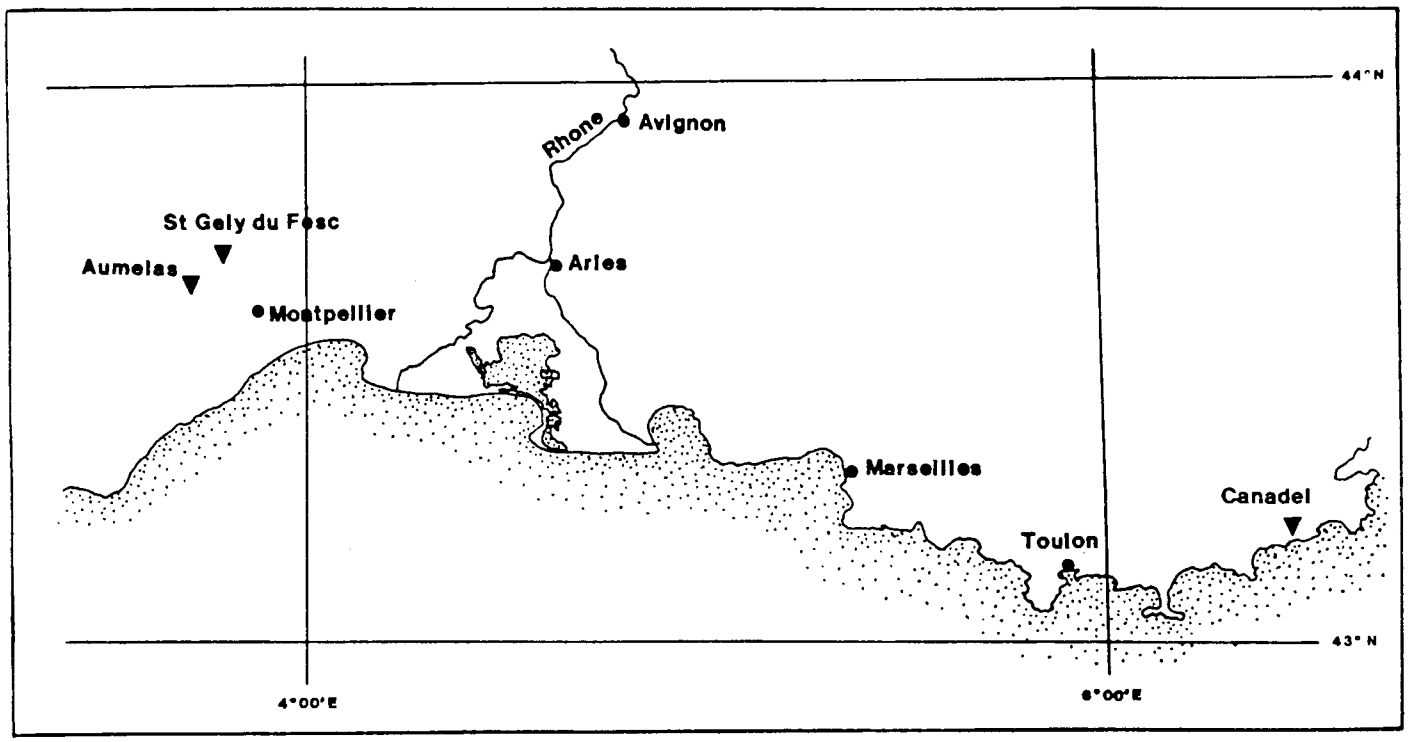


Figure 6.1.1 The location of the two study sites in garrigue (St Gely du Fesc and Aumelas) and the study site in maquis vegetation (Canadel) in southern France.

Appendix 6.1.1. Nitrogen (% d.w.), phosphorus (% d.w.) and water content (%) of leaves of a number of plant species occurring in a) garrigue (Aumelas and St Gely du Fesc) and b) maquis (Canadel) in southern France.

a) Garrigue

Species	Condition	N	P	Water
Aumelas				
Quercus coccifera	Heavily browsed	1.52	0.740	34.0
Q. ilex	"	1.53	0.078	37.4
Phillyrea angustifolia	"	1.70	0.089	49.2
Buxus sempervirens	"	2.23	0.082	35.9
St. Gely du Fesc				
Q. coccifera	Unbrowsed,unburnt,unfertilised	1.44	0.056	37.1
"	"	1.35	0.063	37.7
Q. ilex	"	1.46	0.060	38.4
Q. coccifera	Controlled browsing,fertilised	1.59	0.095	35.6
Q. ilex	"	1.83	0.088	37.2
Q. coccifera	Unbrowsed,fertilised	1.58	0.080	32.7
Q. ilex	"	1.49	0.070	37.4
Q. coccifera	Burnt (2 y frequency)	1.24	0.053	35.5
Q. coccifera	Burnt (6 y frequency)	1.41	0.061	31.7
Pistacia lentiscus	Natural	1.30	0.056	42.5
P. lentiscus	Browsed,fertilised	1.61	0.113	46.8
P. angustifolia	Unbrowsed,unburnt,unfertilised	1.53	0.061	41.3
Cistus monspeliensis	"	1.47	0.087	45.2

b) Maquis

Canadel				
Q. coccifera	Unbrowsed,unburnt,unfertilised	1.29	0.062	38.9
Q. ilex	"	1.45	0.093	47.7
P. lentiscus	"	1.05	0.085	44.5
P. angustifolia	"	1.22	0.075	43.8
C. monspeliensis	"	1.43	0.181	56.4

Appendix 6.1.2. Levels of tannin polyphenols from leaf material of Q. coccifera subjected to different treatments in a latin-square design.

a) Condensed tannins (mg g^{-1} d.w. Quebracho tannin equivalents (QTE))

	Unbrowsed			Browsed		
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n
A extract						
Unfertilised	24.70	11.49	7	31.89	13.32	3
Fertilised	16.46	12.88	4	8.52	7.53	5
A+B extracts						
Unfertilised	30.21	10.71	7	38.45	15.22	3
Fertilised	21.34	14.26	4	10.56	10.15	5

b) Astringency (mg g^{-1} d.w. Tannic acid equivalents (TAE))

A extract						
Unfertilised	52.15	9.45	7	69.70	1.61	3
Fertilised	60.67	5.74	4	54.30	5.93	5
A+B extracts						
Unfertilised	66.77	10.29	7	82.63	6.83	3
Fertilised	75.50	6.81	4	65.67	5.92	5

c) Total polyphenols (mg g^{-1} d.w. (TAE)

A extract						
Unfertilised	45.67	7.96	7	52.70	11.40	3
Fertilised	52.40	6.26	4	41.60	8.66	5
A+B extract						
Unfertilised	60.46	8.84	7	66.53	15.43	3
Fertilised	65.10	6.73	4	55.40	8.85	5

7.0 Oak woodland and Californian chaparral

The mediterranean climate zone of California encompasses at least two dozen vegetation types (Barbour & Major 1977). These occur on a geologically young orogenic substrate with volcanic and earthquake activity. Cycles of crustal uplift and mountain building from the Jurassic to the Quaternary periods have produced the Sierra Nevada mountains as a high eastern boundary and a series of lower coastal ranges sloping to the sea in the west.

In the foothills, the woodland plant community is dominated by evergreen and deciduous Quercus species (Hanes 1977), and in particular by two endemics Q. douglasii (blue oak) and Q. lobata (valley oak). The ground cover is predominantly grass. Two other oak species are characteristic of this community: Q. agrifolia (coast live oak) is scattered in the valleys of the coastal range, while Q. wislizenii (interior live oak) is found in the central valley. Numerous other oak and other woodland species occur regionally in associations with these four oak species throughout the state. This woodland, together with adjacent grasslands, montane forest and chaparral, all form a mosaic with indistinct boundaries, particularly between oak woodland and chaparral. Oak woodland which occurs on deeper, more developed soils often grades into chaparral through scrubby oak thickets (Griffen 1977).

The interaction of fire and climate on Californian vegetation has received a good deal of attention (Mooney 1977, Di Castri & Mooney 1973, Cody & Mooney 1978, Mooney & Conrad 1977, Miller 1983, Mooney 1983), while soil nutrients and nutrient recycling have received much less (Rundel 1983). In a comparison of Italy, Greece and California, Zinke (1973) related elevation to soil-vegetation catenas. He showed that in

these mediterranean zones, both pH and profile development decreased with increasing altitude. In this scheme, annual grasses and herbs tend to occur on , well-developed soils with pH 8.5 at lower altitude, while macchia (in Europe) or chaparral (in California), along with oak woodland, is found on soil with a pH of 7.5 or lower.

In comparison with other mediterranean ecosystems, Californian soils contain less nitrogen but equivalent levels of phosphorus when compared with the mediterranean basin, and less phosphorus than soils in Chile (Figure 4.0.1).

As mentioned in section 6.0, the mediterranean basin has the closest affinities with California of all mediterranean-type ecosystems. One noteworthy similarity is the importance of oaks, Quercus spp., in both these ecosystems. Oaks in general display substantial variation in leaf morphology, yet some of the evergreen oak species in both ecosystem types have similar leaves. For example, Q. coccifera in France closely resembles Q. agrifolia in California in overall shape and degree of sclerophylly.

In California, all of the oaks as far north as San Francisco bay are subject to intense defoliation in an irregular cycle of between 5 and 10 years by Phryganidia californica Packard (Lepidoptera: Dioptidae)(Harville 1955). P. californica is the only genus of a large tropical family whose range extends northwards into the U.S.A. from Mexico. It is thought to have accompanied the northward radiation of oaks and is adapted to the genus as a food source. In a comparative study of photosynthesis and water relations on co-occurring deciduous and evergreen Californian oaks conducted during part of an outbreak of P. californica Hollinger (1983) noted that the dead insects and insect frass production accompanying complete tree defoliation provided an enormous pulse of N and P into decompostion and soil mineralization processes. However, the effects of

such a pulse on tree productivity or reproduction were not explored. The effects of such defoliation on biomass and nutrient loss would presumably be more severe on an evergreen species, such as Q. agrifolia ,than on a deciduous species, such as Q. lobata. This is because Q. agrifolia stores most of its carbohydrate reserves in its leaves (Mooney & Hayes 1973).

In the first of two chapters in this section, variation is examined in the leaf chemical constituents, particularly carbon-containing tannin polyphenols, of two evergreen and three deciduous oak species. The evergreen species were Q. agrifolia Nèe and Q. durata Jeps., and the deciduous species were Q. douglasii Hook. & Arn., Q. kelloggii J.S. Newberry and Q. lobata Nèe . Larval growth of P. californica is compared on these five oak species. The second chapter describes seasonal changes in the same array of constituents in Q. agrifolia and Q. lobata. The significance of these changes is discussed in relation to the progress of the outbreak of P. californica .

7.1 VARIATION IN TANNIN POLYPHENOLS, NUTRITION AND HERBIVORY IN FIVE CALIFORNIAN OAK SPECIES

7.1 INTRODUCTION

A first major ecological study of the role of tannin polyphenols in the interaction of plants and herbivorous insects was that of Feeny (1968, 1969, 1970) and Feeny and Bostock (1968). In their study of winter moth (Operophtera brumata L.) herbivory on pedunculate oak (Q. robur), they suggested that seasonal changes in the balance of oak leaf tannins against beneficial nutrients were the main reason that larvae avoided eating mature oak leaves. Since then, a large amount of research and theory has been published on many types of secondary compounds (e.g. Feeny 1976, Rhoades & Cates 1976, Levin 1976a, Rosenthal & Janzen 1979, Rhoades 1979, 1983). For many structurally simple compounds such as cyanogenic glycosides and alkaloids, both chemical variation and diversity as well as taxonomic differences have been examined and documented (e.g. Dolinger et al 1973, Ikeda et al. 1977, Lincoln & Murray 1978, Robinson 1979, Conn 1979, Whitam & Slobodchikoff 1981). Much less work seems to have been done on tannin polyphenols (Zucker 1983), in part due to their complexity and inextractibility (Zucker 1983, Haslam 1966, 1979, Bate-Smith 1954, 1962, 1969, 1977, Bate-Smith & Metcalf 1957).

Californian oaks, Quercus spp., are heavily defoliated as far north as the San Francisco Bay area in an irregular 5 to 10 year cycle by larvae of the oakmoth, Phryganidia californica (Harville 1955). This species appears to use both deciduous and evergreen oaks indiscriminately (Puttick, unpublished information). Hollinger (1983) examined the relative efficiency of photosynthesis and water relations in a co-occurring pair of deciduous and evergreen oaks, and the defoliation caused by P. californica introduced an important factor into that research. Oak foliage generally contains high levels of tannin polyphenols (Feeny 1970) and levels of as high as 42 % have been

reported for Q. agrifolia in California (Atsatt & Ingram 1983).

The main questions which I sought to answer in this study were: 1) what variation exists in the leaf chemistry of five congeneric co-occurring Quercus species, in particular regarding compounds that are potentially defensive or nutritional for a lepidopteran herbivore; 2) what relationship exists, if any, among the chemical variables which were measured; and, 3) how does leaf chemistry among the three deciduous and two evergreen oaks correlate with larval growth ?

7.1 METHODS

Study site

This study was conducted at Jasper Ridge Biological Preserve, property of Stanford University, 8 km west of Palo Alto, San Mateo County on the San Francisco peninsula, California. Except for Q. durata , isolated individual oak trees which were fully grown with large diameter at breast height (dbh) (> 40 cm), were selected for study. The evergreen species were Q. agrifolia and Q. durata , and the deciduous species were Q. douglasii , Q. kelloggii and Q. lobata. Q. durata generally forms a continuous canopy of lower, stunted-looking plants growing on low nutrient (serpentine) soils, often adjacent to chaparral (Griffen 1977). Nevertheless, it was possible to select and mark individual Q. durata plants.

Collection of leaf material and chemical methods

Approximately 500 g of whole leaves were collected from as many accessible branches

as possible around the whole circumference of 10 individually marked trees of each of the five oak species. The leaf material was sealed in plastic bags and placed in an insulated box with an ice pack. This material was transferred to a laboratory refrigerator for a maximum of 48 hours before further processing. Material was collected in spring (April 26 - 29) and late summer (August 8 - 11).

Eight fresh leaves were individually weighed, the petioles marked for later identification of each leaf, and then photocopied to obtain leaf area. The photocopy of the leaf was weighed along with a known area of photocopy paper, and the area obtained by proportion. The eight leaves were then oven-dried to constant mass and reweighed to measure moisture content. The final dry weight divided by the area of the leaf provided the Leaf Specific Weight (LSW), which is an index of sclerophylly (Mooney & Gulmon 1982).

Another sample of fresh leaf material was extracted for tannin polyphenols using the methods of Swain and Hillis (1959) as outlined in Chapter 5.2. These 100% methanol and aqueous (50%) methanol extracts were tightly sealed and stored at 0 - 4° C until the earliest opportunity for chemical testing. These tests included the "Improved Method" for total polyphenols using Folin-Ciocalteu reagent (Singleton & Rossi 1966), modified for an Auto-analyzer using tannic acid (Sigma Corp.) as a standard (Slinkard & Singleton 1977), and haemanalysis for astringency (Bate-Smith 1973, 1977) using fresh heparinised human blood as the protein chromophore source, with tannic acid as a standard. Finally, samples were analysed for condensed tannins, specifically proanthocyanidins, by hydrolysis with Butanol-HCl (Swain & Hillis 1959, modified by Goldstein & Swain 1963) using Quebracho tannin ("Barktan"®, Van Dyke Supply Company, Woonsocket, South Dakota, USA) as a standard. Both aqueous and pure

methanol extracts were tested with the assays mentioned above. Results for total polyphenols and astringency were expressed as percent tannic acid equivalents (TAE), and for proanthocyanidins as percent quebracho tannin equivalents (QTE) .

The use of a two-tier sequential extraction procedure using pure methanol first, followed by aqueous methanol, separates lower molecular weight phenolics (usually hydrolysable tannins) from the more complex and more tightly bound compounds (usually condensed tannins) (Hillis & Swain 1959). Using a ratio derived from the two extracts to compare concentrations of hydrolysable and condensed tannins from the three different assays provides some information about the relative proportions of simple and complex compounds which may be present in a plant tissue sample. Hypothetically, younger tissue might have higher concentrations oftotal tannin polyphenols in the pure methanol extract (hydrolysable tannins) than in aqueous methanol extract (condensed tannin) when measured using Folin-Ciocalteu reagent.

The remaining fresh leaf material was oven-dried at 60° C to constant mass. The dried material was ground in a Wiley mill through a 20 gauge mesh. Small (approximately 100 mg) samples of this were Kjeldahl block digested in a Technicon block digestor for nitrogen and phosphorus determination. Nitrogen was measured colorimetrically by the indophenol complex formed in a Berthelot reaction and phosphorus by a phospho-molybdate complex. Both complexes were measured at 660 nm using a Technicon Autoanalyzer II which made it possible to process a large number of samples quickly and easily (Technicon methods TA4 - 0323 - 1, 329 - 74 W/B and 146/71 A).

Comparison of larval mass

Phryganidia californica has a bivoltine life cycle, although there are occasional reports where it was trivoltine. The second generation of eggs each year are laid towards the end of summer and the newly hatched larvae overwinter in the second or third instar on the evergreen oaks (Harville 1956). Those larvae on deciduous trees presumably die with leaf fall. Oak moth larvae were separated from leaf samples (20 - 30 leaves) which had been collected from five oak species during late summer for chemical analyses. These individuals had all hatched at the same time from eggs laid approximately 5 - 6 weeks earlier in late June. Each larva was weighed separately, and means were calculated for groups of larvae from each individual tree. Since all had hatched during the same time interval, it was assumed that differences in mass would reflect differences in growth rate.

Statistical analysis

Step-wise multiple regression was used to examine possible correlation of six leaf chemical constituents with LSW as a dependent variable. All six were included in analysis since increased LSW is the complex outcome of a number of different leaf physiological processes which involve both nitrogen and carbon. A series of four of these multiple regression analyses was performed to examine seasonal differences among spring and late summer leaves of the five oak species in order to compare deciduous and evergreen species. Data matrices were assembled comprising values for both nutritional and defensive leaf chemicals. These were nitrogen, phosphorus, and water contents as nutritional components, and measures of total polyphenols, condensed tannins and astringency as defensive chemicals. A seventh set of data was comprised of LSW for each tree sample. To improve the distribution characteristics in the data sets, all percentages were arcsin transformed (Zar 1974). A computer - executed

Step-wise regression program was also used to examine correlations of seven nutritional and 'defensive' leaf components (variables) on larval mass as the dependent variable. The program, STATPRO, was executed on an Apple IIe microcomputer.
Mann-Whitney U tests were used to compare mean larval mass from each oak species to establish if there were between - species differences.

7.1 RESULTS

Variation in the leaf chemical constituents

Total polyphenol levels (tannic acid equivalents TAE) varied from 8.5 % d.w. (Q. kelloggii in late summer) to 27.5 % d.w. (Q. durata in late summer) (Figure 7.1.1a). Although there was no clear distinction between evergreen and deciduous species in the total polyphenol levels, it does appear that the two evergreen species showed increased levels of polyphenols from spring to late summer, while deciduous species showed a decrease (Figure 7.1.1a). Levels of total polyphenols in aqueous methanol extracts were fairly constant (2 - 4 TAE%) in all five species. Condensed tannin levels (quebracho tannin equivalents QTE) in the three deciduous species increased from two to four times (2.5 - 10.0 QTE%) from spring to late summer. Levels in Q. agrifolia increased very slightly, but in Q. durata they were exceptionally high (42.0 QTE%) and then declined by approximately half. The aqueous methanol extracts from the Q. kelloggii contained no condensed tannins, or very low levels. Astringency, as a functional test of tannin activity, did not appear to show any species, seasonal or deciduous/evergreen patterns. There seemed to be a slight decrease in astringency from

spring to late summer in four of the five species, but Q. douglasii showed a 7.0 TAE% increase. Astringency in the aqueous methanol extracts was fairly constant in all five species.

Leaf nitrogen levels were much higher in deciduous than in evergreen species (Figure 7.1.1b). N levels declined slightly from spring to late summer in deciduous species but appeared unchanged, or even rose slightly, in evergreen species. Phosphorus levels were lower in late summer than in spring in the four species for which values are available. Like N, P levels were higher in deciduous species than in evergreens. Leaf specific weight was higher in evergreen than in deciduous species. A given leaf area of Q. durata was approximately twice as heavy as the same area of Q. kelloggii in late summer. LSW in the two deciduous species were approximately 20 % higher in late summer than in spring. Finally, leaf water content appeared to be slightly higher in deciduous than in evergreen species. Leaf water content decreased slightly from spring to late summer in all five species, although this was negligibly small in Q. durata.

Polyphenols in the pure methanol and aqueous methanol extracts.

Ratios for total polyphenols indicated that pure methanol total polyphenols exceeded aqueous methanol total polyphenols 3 - to 7 - fold. In spring, the ratios for deciduous species were somewhat higher than those for evergreens (Table 7.1.1). The ratios of condensed tannins were much more variable and more difficult to interpret both because some values were missing for some of the extracts and also because sample sizes were very different for each species. The ratios for astringency closely approximate those for total polyphenols species by species, except for the high value for Q. durata in spring

which is similar to the ratio for condensed tannins instead.

Polyphenols and nitrogen

Evergreen oaks had a lower protein : tannin ratio than deciduous oaks (Figure 7.1.2). Protein : total polyphenol ratios in deciduous species increased consistently from spring to late summer, while the ratio decreased in evergreen species. Protein : astringency showed exactly the opposite trend except in Q. lobata in which the protein : condensed tannin ratios appeared to parallel the changes in protein : astringency ratios more closely than did the protein : total polyphenol ratios. There was a significant inverse correlation in evergreen oaks between astringency and nitrogen ($r = 0.66$, $n = 36$, $p < 0.001$) (Figure 7.1.3a). There was no significant correlation in deciduous oaks ($r = 0.01$, $n = 57$, $p > 0.1$) (Figure 7.1.3b).

Sclerophylly and leaf chemical constituents

Stepwise multiple regression analyses suggested that combinations of chemical variables correlated with increasing sclerophyllization as leaves aged through the season. A series of correlation matrices showed that LSW correlated positively with different tannin measures during late summer in all the oaks and in evergreens irrespective of season (Table 7.1.2a). Of the three tannin measures, only condensed tannins correlated positively with LSW in all oaks in both seasons. N, P and water content were all negatively correlated with LSW in all oaks in both seasons. Different combinations of independent variables were found to be good predictors of LSW (Table 7.1.2b). In general, calculated F values should be greater than the tabulated values by four times or more in order for a variable to be considered a reliable predictor (Draper & Smith 1981).

In spring, leaf water content was the strongest single predictor of LSW, and was inversely correlated with LSW, for all oaks and in both seasons for deciduous oaks. In late summer, however, total polyphenols, which were positively correlated with LSW, became the strongest predictor of LSW. Nitrogen was a second negatively correlated predictor in both spring and late summer. The single best predictor for LSW in evergreen oaks was astringency, which was also positively correlated with LSW.

Oak moth larval development on different oaks

The mean weights of larvae collected from evergreen oaks were significantly lower than those of larvae collected from deciduous species (Table 7.1.3, Table 7.1.4). Step-wise multiple regression analyses indicated that P and astringency show highest correlations with larval mass (Table 7.1.5a), and these two variables coupled together make a significant contribution to a predictive equation(Table 7.1.5). However, the F values for these two independent variables, although significant, were not high enough for them to be considered strong predictors of larval mass.

7.1 DISCUSSION

The results of this study have shown that five congeneric oak species have very different combinations of leaf chemical components. This variation appeared to be seasonally consistent within the five species although there were insufficient data to examine seasonal changes rigorously: this topic is explored in Chapter 7.2 for two of the oak species in this study. To my knowledge, information about variation in polyphenols in so many congeneric and co-occurring species has not been published elsewhere. Within the range of variation found in this study, it was still possible to distinguish evergreen oaks from deciduous oaks, using leaf chemical characteristics. These differences in chemical constituents were reflected in the growth rates of P. californica larvae in a "natural" bio-assay. Larvae on the evergreen oaks had significantly lower growth rates than those on the deciduous oaks. The results of stepwise regression analysis using larval weights as the dependent variable and nitrogen, phosphorus, moisture, leaf specific weight and three measures of tannin polyphenols as independent variables, proved interesting. The two best predictors were found to be phosphorus (positively) and astringency (negatively). However, when coupled together into a predictive regression equation, they were only marginally significant statistically. The importance of phosphorus in insect nutrition appears to have received very little attention and certainly warrants further experimental investigation. The importance of astringency is supported by some laboratory experiments where increased concentration of tannic acid in artificial diet significantly reduced the growth rates of P. californica larvae (Puttick & Glyphis, unpublished data). In that experiment, a threshold tannin concentration of 12% was found above which larval growth was depressed. Tannin concentrations as high as 25 % were recorded in the five oak species, which certainly suggests that these defensive chemicals would adversely affect the growth of oak moth larvae. Very few studies have been published which have demonstrated dose dependent effects of

secondary chemicals, and particularly of tannin polyphenols. The chemical interplay in insect nutrition of beneficial (e.g. protein, fats) and toxic chemicals (e.g. tannins, alkaloids) is undoubtedly complex (Feeny 1969, 1970,) and this has led to some debate regarding the relative importance of each (Slansky & Feeny 1977,). A similar debate exists in vertebrate nutritional/ecological studies as outlined in the discussion in Chapter 5.3 (Bryant & Kuropat 1980, and references therein).

The phenomenon of variation in host plants has recently been extensively reviewed (Denno & McClure 1983). Aside from the actual presence or absence of defensive chemicals in plants, variation in the concentration of these chemicals, and in the concentration of nutritional compounds, represents a major defence barrier for insects (Southwood 1973, Mattson & Addy 1975, Denno & McClure 1983). The inter-specific differences among the five oaks described in this study may represent such a level of variation acting as a defence against the oak moth P. californica , a specialist on the genus Quercus . This is not to say that the variation evolved as a defence against herbivores; it may have been present as part of the variation to be expected in such a congeneric assemblage, with each species adapted to its own edaphic and environmental conditions. The occurrence of Californian oaks on a low nutrient substrate may account to some extent for higher levels of polyphenols and, therefore, the wide range in polyphenol concentrations in oak foliage. Low levels of nitrogen in the substrate have been correlated with increases in the biosynthesis of tannin polyphenols from both experimental evidence for single plants (sunflowers Helianthus annus)(Del Moral 1972) and observational evidence for a number of species in two different plant communities (Davies et al. 1964, McKey et al. 1978). A comparison of a few oaks from both North American and European ecosystems with oaks from mediterranean ecosystems indicates that higher levels of polyphenols occur in the mediterranean species (Table 7.1.6).

The nitrogen values obtained in this study were converted to protein using a standard factor (6.25). However, as mentioned in the discussion in Chapter 5.3, this factor is derived from the proportion of nitrogen in a protein molecule (approximately 16%) which may vary among different kinds of proteins (Robbins 1983, Milton & Dintzis 1981). Proteins and polyphenols have different affinities for each other in forming complexes (Van Sumere et al. 1975), and the ratios of interacting molecules can vary over several orders of magnitude (Hagerman & Butler 1980). In addition, specific proteins may only be complexed by specific polyphenols depending on, among other factors, available binding sites, pH, polymer sizes and three dimensional structure (Hagerman & Butler 1981). In this study, a ratio of 1 : 1 for tannin : polyphenol concentrations was chosen based on experiments with oak tannin complexing with casein (Feeny 1969) although this may not be the correct ratio for the oak tannins and proteins in the Californian oak species. Nevertheless, in using these ratios I showed that evergreen species were quite different from deciduous species (Figure 7.1.2). The much lower nitrogen levels in evergreen leaves (Figure 7.1.1b) coupled with a significant astringency - nitrogen relationship (Figure 7.1.3a) suggested that polyphenols may protect this N from consumption by larvae, at least in non-outbreak years, as the level of N approached a lower minimum concentration of approximately 1 %. The absence of a significant astringency - nitrogen relationship in deciduous species (Figure 7.1.3b), and their higher levels of N (Figure 7.1.1b), imply that deciduous species are not faced with this problem even though they were also very heavily or even completely defoliated. This is also consistent with the finding that most of the carbohydrate reserves in Q. agrifolia were contained in the leaves (Mooney & Hays 1973). The authors suggested that this rendered the oak more vulnerable to the effects of defoliation than a deciduous species, Aesculus californica, which stored its carbohydrate reserves throughout the plant. In another study, evergreen arctic shrubs showed a greater reduction in growth than deciduous shrubs when artificially defoliated (Archer & Tieszen

1980, Chapin 1980b).

Sclerophyllous leaves are a notable characteristic of shrubs in mediterranean-type ecosystems (Schimper 1903, Mooney & Dunn 1970, Dement & Mooney 1974). Different factors have been implicated in the evolution of sclerophylly, including water shortage (Wood 1933, Grieve 1955, Cunningham & Strain 1969, Orians & Solbrig), low soil nutrients(Loveless 1961, Beadle 1966, Monk 1966, Small 1972, Chapin 1980a) and herbivory (Mooney & Dunn 1970, Reader 1979, Rauscher 1981). The development of sclerophylly during growth of a leaf involves obvious physical changes particularly in LSW, as well as changes in lignification (Swain1979), nitrogen and carbon (Mooney & Gulmon 1982), phosphorus (Beadle 1966) and other nutrient compounds (Chapin 1980a). In this study, the results of stepwise regression analysis on LSW as an index of sclerophylly, and nitrogen, phosphorous, moisture and the three measures of tannin polyphenols as independent variables, suggested that different variables in evergreen and deciduous species were correlated with LSW. In deciduous oaks, a decline in leaf moisture content correlated most closely with increased LSW, while in evergreen leaves, the strongest correlations with LSW were polyphenols and astringency. This suggests that the sclerophyllization process in evergreens may be linked in some way to polyphenol biosynthesis, possibly through lignification and the Shikimate pathway (Swain 1979), while in deciduous oaks the physical process of dehydration as the leaves age seems to be more important.

In summary, concentrations of tannin polyphenols varied widely among the five oak species, and the growth of oak moth larvae was significantly affected both by the concentration of phosphorus and the degree of astringency of the leaves.

Table 7.1.1. Ratios of polyphenols in pure methanol to polyphenols in aqueous (50 %) methanol in extracts from five Californian oak species in two seasons as measured by Folin-Ciocalteu test (Total polyphenols), Butanol - HCl hydrolysis (Proanthocyanidins) and Haemanalysis (Astringency).

		Total polyphenols			Proanthocyanidins			Astringency		
		$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n
Q. agrifolia	Spring	4.50	1.88	14	1.14	-	1	5.17	1.89	13
	Autumn	3.40	0.51	11	1.34	0.55	3	3.56	0.76	6
Q. douglasii	Spring	7.72	1.52	10	-	-	-	4.81	2.16	10
	Autumn	4.12	1.07	11	3.67	3.06	3	5.47	1.16	10
Q. durata	Spring	4.27	0.66	14	13.23	6.26	10	12.44	8.89	14
	Autumn	5.16	1.00	10	9.95	0.89	10	6.54	1.30	10
Q. kelloggii	Spring	5.62	1.99	10	1.60	-	1	9.02	2.49	10
	Autumn	3.63	0.50	7	0.82	-	1	6.31	0.83	7
Q. lobata	Spring	6.07	0.88	10	-	-	-	6.99	1.76	10
	Autumn	4.38	1.09	10	5.38	1.84	7	5.39	1.53	10

Table 7.1.2a. Partial correlation coefficient tables generated from stepwise regression analyses of leaf specific weight as a dependent variable and six leaf chemical characteristics as independent variables in five species of Californian oak collected and tested in two seasons.

	N	P	H_2O	Tot Poly	Astring	Proanth
SPRING (All Oaks)						
Phosphorus	0.91					
Water content	0.70	0.71				
Total polyphenols	0.60	0.53	0.73			
Astringency	0.53	0.52	0.54	0.50		
Proanthocyanidins	-0.14	-0.27	-0.23	-0.13	0.00	
Leaf specific weight	-0.82	-0.81	-0.82	-0.68	-0.64	0.04
AUTUMN (All Oaks)						
Phosphorus	0.57					
Water content	0.41	0.32				
Total polyphenols	-0.19	-0.44	-0.26			
Astringency	0.18	0.05	0.11	0.55		
Condensed tannins	0.04	-0.36	-0.23	0.56	0.58	
Leaf specific weight	-0.55	-0.52	-0.49	0.71	0.26	0.41
DECIDUOUS OAKS (Spring and Autumn)						
Phosphorus	0.61					
Water content	0.50	0.68				
Total polyphenols	0.48	0.50	0.68			
Astringency	0.05	0.01	0.15	0.31		
Condensed tannins	-0.37	-0.79	-0.62	-0.35	0.13	
Leaf specific weight	-0.25	-0.47	-0.68	-0.22	-0.07	0.51
EVERGREEN OAKS (Spring and Autumn)						
Phosphorus	0.35					
Water content	0.34	0.76				
Total polyphenols	-0.08	-0.54	-0.28			
Astringency	-0.12	-0.49	-0.46	0.54		
Condensed tannins	-0.31	-0.71	-0.71	0.67	0.75	
Leaf specific weight	-0.23	-0.52	-0.52	0.56	0.75	0.71

Table 7.1.2b. Results of stepwise multiple regression analysis on data from Table 7.1.2a. For all oaks in spring $df_1 = 6$, $df_2 = 26$, $F_{0.05} = 2.47$, $F_{0.01} = 3.82$. For all oaks in autumn $df_1 = 6$, $df_2 = 29$, $F_{0.05} = 2.43$, $F_{0.01} = 3.50$. For deciduous oaks $df_1 = 6$, $df_2 = 40$, $F_{0.05} = 2.34$, $F_{0.01} = 3.29$. For evergreen oaks $df_1 = 6$, $df_2 = 15$, $F_{0.05} = 2.79$, $F_{0.01} = 4.32$.

Dependent variable	F	Partial F	Partial r	R^2	SD of resid.	SE as % of means	B coeff.
Spring (all oaks)							
Step 1 Water content	65.94	14.10	-0.82	0.68	0.0024	18.20	-0.0005
Step 2 Nitrogen	58.32	13.44	-0.82	0.80	0.0019	14.80	-0.0013
Step 3 Astringency	42.75	3.25	-0.64	0.82	0.0019	14.30	-0.0002
Constant							0.0505
Autumn (all oaks)							
Step 1 Total polyphenols	34.80	36.40	0.71	0.51	0.0032	21.70	0.0007
Step 2 Nitrogen	35.93	11.90	-0.55	0.69	0.0026	17.60	-0.0019
Step 3 Water content	26.49	3.10	-0.49	0.71	0.0025	17.07	-0.0003
Constant							0.0276
Deciduous oaks (both seasons)							
Step 1 Water content	38.04	52.20	-0.68	0.46	0.0019	16.30	0.0003
Step 2 Total polyphenols	28.64	10.90	-0.22	0.57	0.0017	14.70	-0.0006
Constant							0.0312
Evergreen oaks (both seasons)							
Step 1 Astringency	25.38	25.38	0.75	0.56	0.0027	14.60	0.0007
Constant							0.0037

Table 7.1.3. Mean weights (grams) of oak moth larvae collected from five oak species. N = total number of larvae collected from all trees.

Oak species	Larval weight (g)		N
	Mean	S.E.	
Quercus agrifolia (11 trees)	2.66	0.20	127
Q. lobata (10 trees)	3.53	0.25	197
Q. douglasii (11 trees)	4.26	0.23	223
Q. kelloggii (7 trees)	3.04	0.17	151
Q. durata (8 trees)	2.04	0.15	42

Table 7.1.4. Significance levels of differences among mean weights of P. californica larvae collected from individual oaks (Mann-Whitney U tests).

n =	Q. agrifolia 11	Q. kelloggii 7	Q. douglasii 11	Q. lobata 10	n
Q. kelloggii	**				7
Q. douglasii	***	N.S.			11
Q. lobata	**	N.S.	N.S.		10
Q. durata	N.S.	***	***	*	8

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

N.S. $p > 0.1$

Table 7.1.5a. Correlation matrix between food quality and P. californica mean weight.

	N	P	LSW	H_2O	Tot. Poly.	Astring.	Proantho cyanidins
P	0.53						
LSW	-0.56	-0.50					
H_2O content	0.31	0.37	-0.42				
Tot.poly.	-0.18	-0.47	0.70	-0.29			
Astringency	0.18	-0.04	0.25	-0.01	0.57		
Proanthocy.	0.11	-0.38	0.33	-0.35	0.54	0.57	
Larval weight	0.04	0.30	-0.07	0.29	-0.18	-0.29	-0.17

Table 7.1.5b. Stepwise multiple regression of P. californica larval weight on food quality of Californian oaks. $df_1 = 7$, $df_2 = 30$, $F_{0.01} = 3.30$

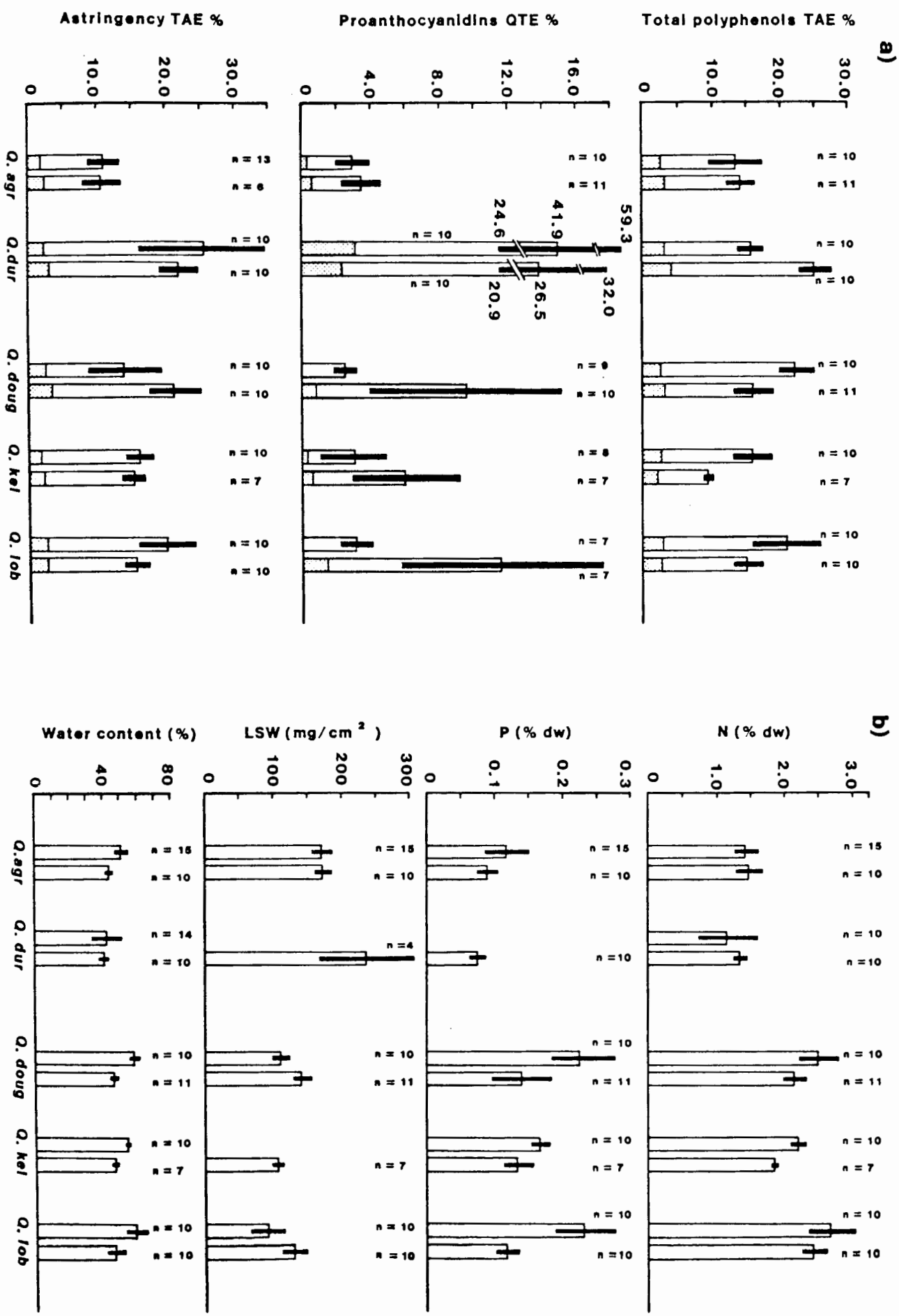
Dependent variable	F	Partial F	Partial r	R^2	SD of resid.	SE as % of mean	B coeff.
Step 1 Phosphorus	3.50	3.48	0.30	0.09	15.1	262.5	14.56
Step 2 Astringency	3.52	3.31	-0.29	0.17	14.6	254.5	-1.11
Constant							3.90

Table 7.1.6. The range of total polyphenol levels (tannic acid equivalents(TAE) % d.w.) in several oak species from Europe and North America.

Species	Total polyphenols (TAE % d.w)	Sources
EUROPE		
Quercus robur (Pedunculate oak)	0.66 - 5.50	Feeny 1970
Q. coccifera[1] (Kermes scrub oak)	7.48 - 12.77	This study (Chapter 6.1)
Q. ilex[1]	2.47 - 9.60	"
N. AMERICA		
Q. rubra (Red oak)	3.40 - 9.20	Schultz & Baldwin 1982
Q. agrifolia[1] (Coast live oak)	7.54 - 19.46	This study (Chapter 7.1)
Q. durata[1] (Leather oak)	15.50 - 26.96	"
Q. kelloggii[1] (Black oak)	8.09 - 18.62	"
Q. douglasii[1] (Blue oak)	14.26 - 28.15	"
Q. lobata[1] (Valley oak)	11.55 - 27.76	"

[1]Mediterranean species.

Figure 7.1.1 A comparison of a) total polyphenols, proanthocyanidins and astringency and b) nitrogen, phosphorus, leaf specific weight and moisture content in five species of Californian oaks in spring(left hand histogram of the pair) and in late summer(right hand histogram). Vertical bars indicate ± one standard deviation. Shaded areas in each histogram indicate concentrations of polyphenols in the aqueous methanol extract as a portion of the total.


a)
Astringency TAE %
Proanthocyanidins QTE %
Total polyphenols TAE %
Q. agr
Q.dur
Q. doug
Q. kel
Q. lob
b)
Water content (%)
LSW (mg/cm²)
P (% dw)
N (% dw)
Q.agr
Q. dur
Q. doug
Q. kel
Q. lob

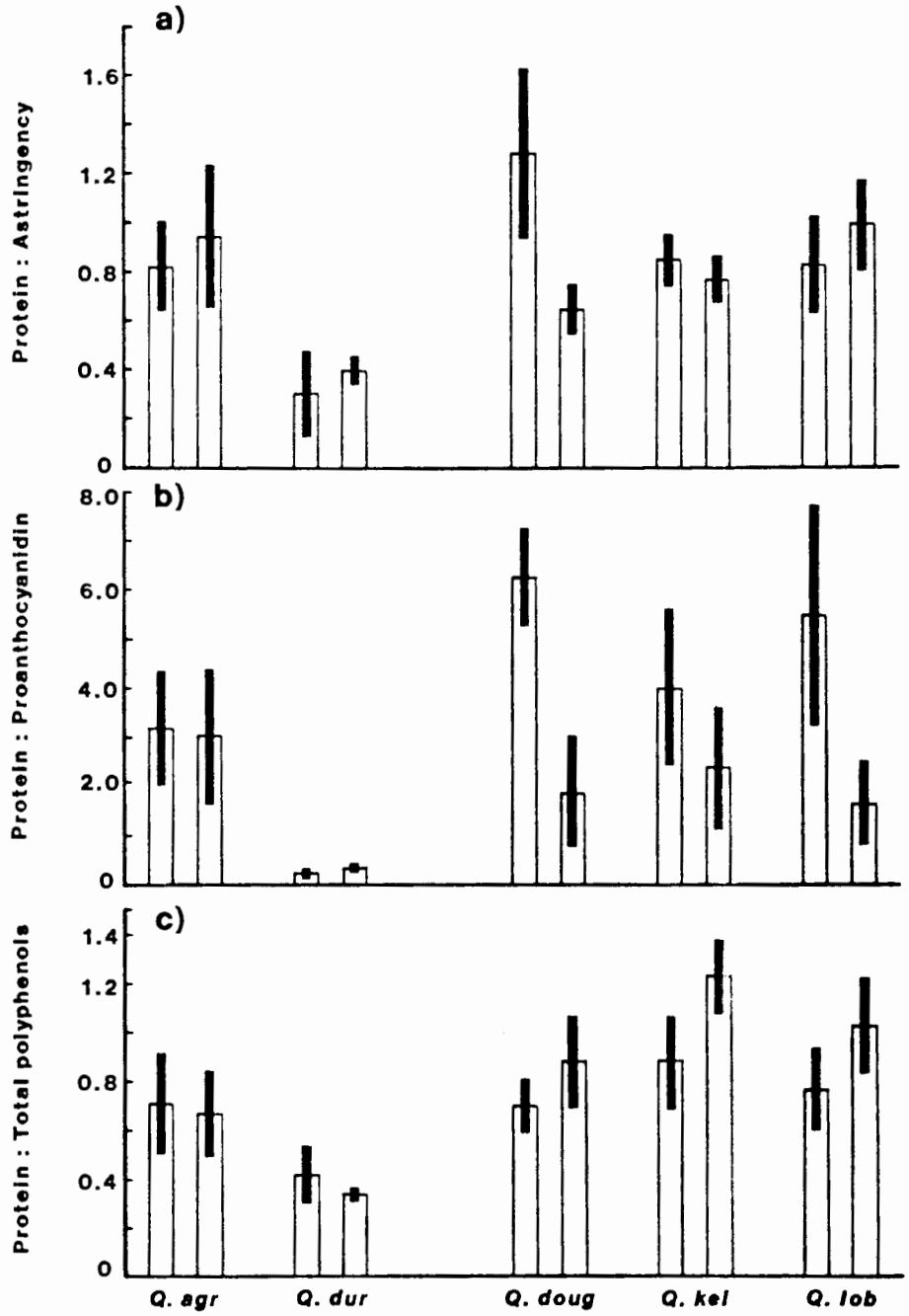


Figure 7.1.2 Ratios of a) protein : astringency b) protein : proanthocyanidins and c) protein : total polyphenols in five species of Californian oaks in spring(right hand histogram of pair) and in late summer(left hand histogram). Vertical bars indicate ± one standard deviation. Protein was calculated from (N (%) x 6.25).

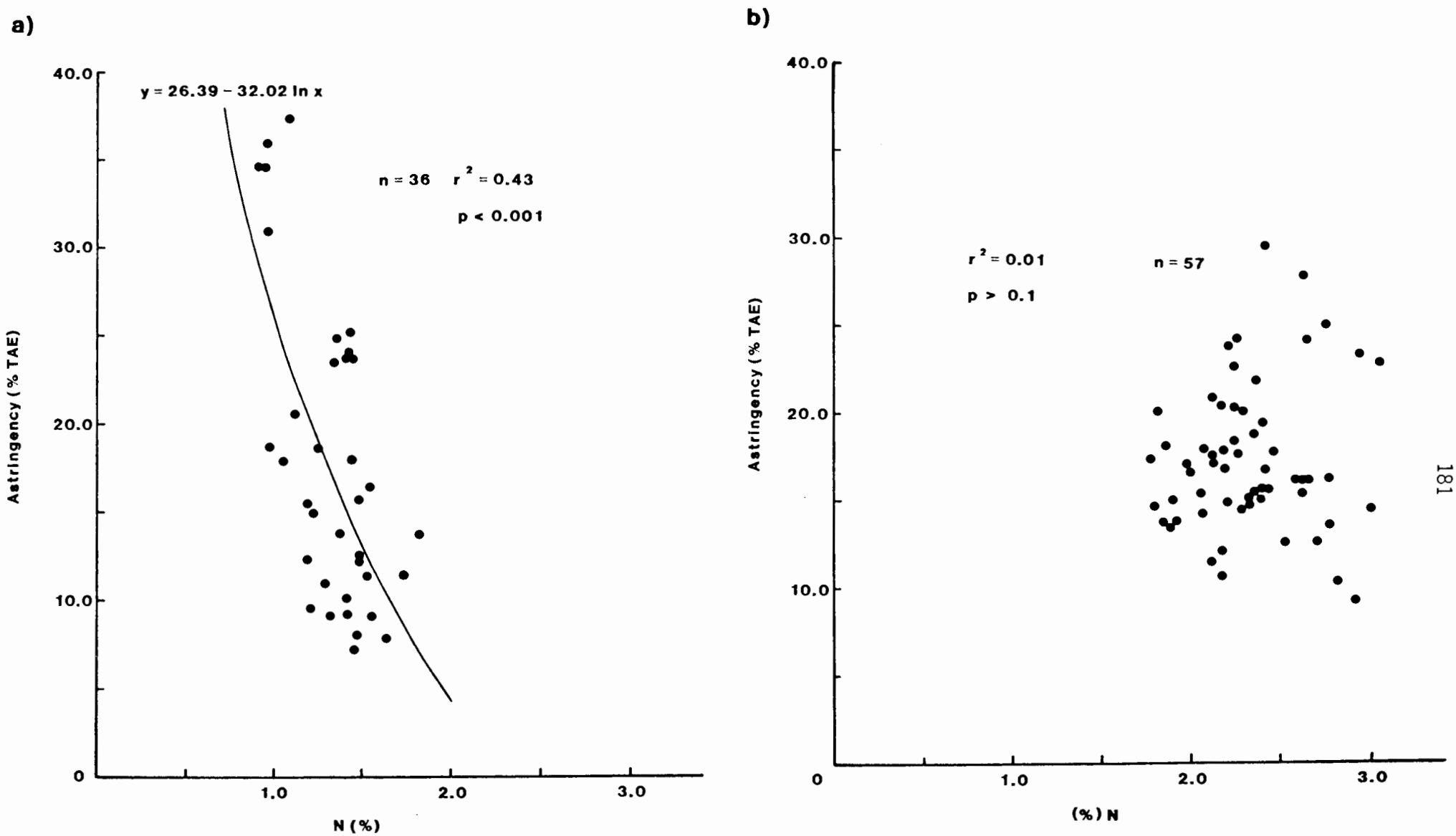


Figure 7.1.3 Relationship between astringency (% TAE d.w.) and nitrogen (% d.w.) in a) evergreen oaks and b) deciduous oaks both in spring and late summer.

7.2 TEMPORAL PATTERNS OF TANNIN POLYPHENOL LEVELS, NUTRIENTS AND HERBIVORY IN TWO CALIFORNIAN OAKS

7.2 INTRODUCTION

The phenomenon of outbreaks in vertebrate and invertebrate populations has been extensively examined (e.g. Chitty 1960, Chitty et al. 1968, Krebs et al. 1973, Keith 1983, Fox & Bryant 1984, Morris 1963, Dempster 1963, Baltensweiler 1968, Kemp & Simmons 1979, May 1975, Noy-Meir 1975, Hassell & May 1973). Many controlling factors have been postulated which include low food nutritional levels (White 1974, 1978, 1984, Mattson & Addy 1975), secondary metabolites (Haukioja & Hakala 1975, Bryant 1984), climate (Klomp 1968, but see White 1978), pollution (Mattson & Addy 1975) and parasitoids (Hassell 1978), among others.

An outbreak of Phryganidia californica in the San Francisco Bay area first became apparent in 1980. These larvae extensively defoliated a number of Californian oak species and completely defoliated individual trees locally in 1981. In spite of complete defoliation,these trees subsequently grew new cohorts of leaves. The pattern of defoliation, of inter- and intra specific variation in different chemical compounds in oak leaves (Chapter 7.1) and the history of P. californica outbreaks in California (Harville 1956), suggest that the oak moth is able to cope, to some extent, with the chemical defences which occur in oaks. Oaks in general contain high levels of tannin polyphenols (Chapter 7.1). For oaks in mediterranean ecosystems, low soil fertility as an edaphic characteristic may separately be responsible for higher levels of tannins than those found in plants occurring on more fertile soils (Davies et al. 1964, McKey 1968, Barry & Forss 1983). However, tannin polyphenols as digestibility-reducing compounds should be effective against generalist insect herbivores, as well as specialists such as P. californica (Rhoades & Cates 1976, Zucker 1983).

The irregular intervals of 5 - 10 years between P. californica outbreaks, in conjunction with climate cycles (Harville 1955), suggest that both food quality, as a combination of nutritional and deterrent compounds, and climate may be the important controlling factors in this cycle (Rhoades 1983). However, a first step to proving such an hypothesis would be to find significantly higher concentrations in the levels of secondary compounds (in this case tannins) above the levels in non-outbreak years i.e. an inductive effect (Rhoades 1983). Second, it would be important to show that such levels of secondary compounds measurably reduce fitness of the defoliating herbivore.

In this study, levels of nitrogen, phosphorus and tannin polyphenols were regularly measured for a period of 15 months in an evergreen oak species, Quercus agrifolia and a deciduous species, Q. lobata , during an outbreak of P. californica . The results from this study are discussed in terms of leaf age and chemical composition during progressive defoliation by P. californica.

7.2 METHODS

Study site

Study trees were situated at the Stanford University Biological Preserve, Jasper Ridge, 8 km west of Palo Alto, San Mateo county, California. Both Q. agrifolia and Q. lobata co-occur in the coastal ranges of California (Figure 7.2.1). They both occur in single species or mixed species stands with a continuous canopy or as single individuals. Single individuals were selected for study to prevent oakmoth larvae transfering from one tree to another thus causing unaccountable population fluctuations of these larvae in a given tree.

Herbivory assessment

Three independent observers estimated the extent of crown defoliation visually on each of the study trees simultaneously. The average of these three estimates was recorded as per cent defoliation for each study tree. These estimates were collected every two weeks for the first four months of the study (May - August 1981) which corresponded to the major period of defoliation by oak moth larvae.

Plant collection and chemical methods

Leaves were collected from four tagged Q. lobata and four tagged Q. agrifolia every two weeks for four months and then monthly for an additional ten months, beginning in late April 1981 and ending in June 1982. All further leaf processing and chemical analysis was completed as outlined in methods in Chapter 7.1.

Stepwise multiple regression analysis

Stepwise multiple regression was used to examine the correlation of six chemical components (independent variables) of the leaves of each of the oak species, and the leaf specific weight (LSW) as a dependent variable of the leaves. Data matrices were assembled from chemical data from individual tree samples collected regularly for the 15 month duration of this study. The multiple regression is part of a STATPRO program run on an Apple IIe microcomputer.

7.2 RESULTS

Defoliation

During April to September 1981, Q. agrifolia trees were defoliated twice by Phryganidia californica larvae : once in late June and again in September (Figure 7.2.2). This meant that some trees produced three cohorts of leaves during 1981. Two of the trees (Figure 7.2.2) had already suffered fairly heavy defoliation at the beginning of this study in April 1981 because P. californica overwintered on these evergreen oaks in the second or third instar. The larvae rapidly increase in weight as temperatures increased from March onwards. Deciduous Q. lobata showed a much more regular pattern of defoliation because of the simultaneous growth of new leaves and the absence of overwintering larvae (Figure 7.2.2). Most of the Q. lobata were almost completely defoliated by September. Some managed to produce a new cohort of leaves before leaf abscission in autumn. One tree (L6) showed a decline in herbivory : this was an artifact of observation due to the simultaneous growth of replacement leaves. Although only four trees of each species were sampled, the level and temporal pattern of defoliation were typical for all oaks at Jasper Ridge.

Tannin polyphenols

Total polyphenol levels were highest in new Q. agrifolia leaves, and appeared to decline thereafter (Figure 7.2.3). This pattern was complicated by the repeated, almost complete defoliation of the study trees over the duration of the study (Figure 7.2.2), as mentioned above. Highest mean levels occurred in spring of both years (20 - 22 %

Tannic acid equivalents (TAE) d.w.) and declined to an overall low of 11 % TAE at the end of winter (February and March 1982). The same pattern was also observed in the levels of total polyphenols in Q. lobata (Figure 7.2.4). New leaves in April 1981 contained a mean concentration of 18.5 % TAE d.w. and this declined to a minimum of 12 % TAE by the end of summer. Overall, Q. lobata was not as heavily defoliated as Q. agrifolia although by the end of summer some individuals were stripped. The apparent increase in total polyphenols in November 1981 can be ascribed to new growth on trees which managed to reflush new leaves just prior to seasonal leaf abscission in late November. Some of these new leaves did persist through winter and the single value indicated for March 1982 (Figure 7.2.4) suggests that total polyphenols may not have changed during this period. New leaves in May contained almost 25 % TAE d.w. total polyphenols declining to 16.5 % TAE in June, which was within 1.5 % TAE of the level for the preceding year.

Astringency in Q. agrifolia leaves followed the same overall seasonal pattern as total polyphenols : it was highest in spring (15 % TAE d.w.) and declined through summer to a minimum of 8.5 % TAE d.w. in March 1982 (Figure 7.2.5). As with total polyphenols, the seasonal pattern was complicated by the complete defoliation of study trees at least twice between June and October 1981. In contrast, astringency in Q. lobata leaves was high in May (22.5 % TAE), declined during the following six-week period to a seasonal minimum of 13 % TAE, but then gradually increased to 18 % during the next four months until leaf abscission (Figure 7.2.6). This gradual increase did not seem to coincide with changes in either total polyphenols or condensed tannins. Polyphenols were declining during this period while condensed tannins were at a raised plateau.

Finally, condensed tannins in Q. agrifolia increased from April to a maximum of 7 % quebracho tannin equivalents (QTE) d.w. in May 1981, declined to a minimum in early July (2 % QTE d.w.), after which they increased steadily to an overall maximum of 10.5 % QTE d.w. in November (Figure 7.2.7). The peak in May 1981 seems to have been repeated in April 1982 after a precipitous decrease in levels during mid-winter. There did not appear to be any obvious pattern to these changes within each new cohort of leaves as it was subsequently consumed. Condensed tannin levels in Q. lobata fluctuated between 2 - 5 % QTE d.w. from month to month during April, May and June 1981 (Figure 7.2.8). In July these levels increased to a higher plateau where they fluctuated between 9 - 12 % QTE d.w. until leaf abscission in November.

Comparison of tannin polyphenol levels in new leaves from the same trees in two successive years in order to establish if there was a long - term inductive effect showed no significant increases (Table 7.2.1). One significant difference was found, however, in astringency in Q. lobata. In this case, astringency in the second year (1982) was significantly lower than in the first year (Paired t-test $t = 5.39$, $df = 8$, $p < 0.0001$).

Nitrogen, phosphorus and moisture

In Q. agrifolia, leaf N levels were high in new leaves (3.5 - 4.0 % d.w.) and declined to a seasonally constant mean of 1.5 % d.w. (Figure 7.2.9). Nitrogen levels in new leaves of Q. lobata were also higher at approximately 3 % d.w. but declined to a higher plateau than N levels in Q. agrifolia . Phosphorus levels in Q. agrifolia changed in much the same way as N, but at levels of one-tenth those of N (0.1 - 0.2 % d.w.) (Figure 7.2.10). In Q. lobata P levels declined in a similar pattern to N but the final plateau level was 0.1 % DW. Leaf moisture content in Q. agrifolia did not show any

obvious seasonal pattern (Figure 7.2.11). However, Q. lobata leaf moisture was high in young leaves (55 %) and then declined to approximately 45 % in mid-summer. The apparent increase in moisture thereafter can probably be attributed to the ending of the mid-summer dry season in late July coupled with the regrowth of new leaves to replace those being defoliated by the oak moth larvae.

Leaf specific weight (LSW)

Increases in LSW in Q. agrifolia occurred most rapidly early in the season but this rate appeared to diminish in successive defoliations by oak moth larvae (Figure 7.2.12). The biggest increase in LSW in Q. lobata occurred in the new leaves during May and June, after which it stayed relatively constant for the remainder of the season (Figure 7.2.12). The correlation matrix comparing LSW in Q. agrifolia with six independent leaf chemical variables suggested that N was significantly inversely correlated (Table 7.2.2a). Only condensed tannins were positively correlated, and then only slightly. Stepwise analysis suggested that N was a significant inverse primary predictor of LSW followed by moisture (Table 7.2.2b). These two variables together accounted for 63 % of the correlation with LSW.

The correlation matrix for Q. lobata LSW indicates a slightly different arrangement of the most significant independent variables compared with Q. agrifolia. In Q. lobata total polyphenols had the highest partial correlation coefficient (-0.73) followed by moisture and N (Table 7.2.3a). However, the contribution of the latter two variables to the multiple correlation coefficient was reversed in the stepwise procedure: N preceded moisture (Table 7.2.3b). These three variables together accounted for 78 % of the correlation with LSW in Q. lobata.

Relationship between protein/nitrogen and tannin polyphenols

In Q. agrifolia, the ratio of protein : polyphenol followed the same general seasonal pattern as the three polyphenol measures did (Figures 7.2.13a, 7.2.13b, 7.2.13c). Any change in this ratio could be attributed to independent increases and decreases in protein or polyphenol. In both total polyphenols and astringency, the ratio was above 1.0 (i.e. where % protein > % polyphenol) between December and March. In total polyphenols the mean fluctuated below 1.0 until December, while in astringency the S.D. ranges straddled 1.0 The protein : astringency ratio showed a steady increase from September through to March. Protein : condensed tannin appeared to have been highest in June and July and decreased to a minimum from August to November.

The seasonal patterns of three protein : polyphenol ratios in Q. lobata also followed a similar general trend to each other but was quite different from that in Q. agrifolia (Figures 7.2.14a, 7.2.14b, 7.2.14c). Protein : total polyphenols increased to a maximum in late June and then declined steadily through the rest of the season (Figure 7.2.14a). Protein exceeded total polyphenols from April to September. The single value given for March 1982 represents old leaves from the previous year which had persisted through winter. Proteins exceeded condensed tannins by the biggest margin in April 1981 but thereafter the ratio declined steadily to a minimum in September. There was a brief increase during October and November before leaf fall. The leaves in May 1982 once again had high ratios (4.0 - 7.0) which declined steeply to a mean of 2.5. Protein : astringency followed the same general course as protein : total polyphenols. Protein exceeded astringency from early June to late August, although with a sharp decline in late July. The single point in March 1982 (Figure 7.2.14) represents a ratio

for leaves which had persisted through the winter on a single tree.

There were significant positive correlations between N and total polyphenols ($r = 0.59$, $n = 17$, $p < 0.01$) and between N and astringency ($r = 0.87$, $n = 17$, $p < 0.001$) in the new leaves of Q. agrifolia (Figures 7.2.15a &7.2.15c), while there was no significant correlation between N and condensed tannins ($r = 0.28$, $n = 17$, $p > 0.1$) (Figure 7.2.15b). However, in the new leaves of Q. lobata (Figures 7.2.16a,b,c), there were no significant correlations between N and total polyphenols ($r = 0.14$, $n = 10$, $p > 0.1$), condensed tannins ($r = 0.13$, $n = 10$, $p > 0.1$) or astringency ($r = 0.1$, $n = 10$, $p > 0.1$).

7.2 DISCUSSION

In this study, polyphenol levels, nitrogen and phosphorus were highest in new leaves and declined as the leaves expanded and aged. Similar patterns, particularly of high polyphenols in young leaves, have been described recently for a number of other plant species (McKey 1979 and references therein, Milton 1979, Gartlan et al. 1980, Oates et al. 1980, Coley 1983, Cooke et al. 1984) contrary to the predictions of a theory of tannins and defence proposed by Rhoades & Cates (1976) and Feeny (1976). These authors made a prediction, among others, that tannin levels should be low in new leaves and should increase as the leaves matured through the season. However, both the plants and herbivores in the studies quoted above exist in very different community structures and have very different life cycles from those on which the original theory was developed. The studies were all conducted at tropical sites with relatively low soil nutrients, whereas the theory was developed from observations from temperate systems. Similarly, Californian oaks in this study also occur on a substrate which has characteristically low soil nutrients. This apparent correlation clearly warrants further investigation. However, Phryganidia californica utilizes both life forms in its bivoltine life cycle and oak moths show no significant difference in preference between Q. agrifolia and Q. lobata for oviposition sites (Puttick, unpublished data). This means that the overwintering second instar larvae on Q. agrifolia leaves would begin eating the cohort of leaves just as soon as these were available in the spring. Therefore it would be especially important for the new Q. agrifolia leaves to be well-defended as early as possible. It is also conceivable that, since Q. lobata often occurs in a continuous canopy with Q. agrifolia, there could be migration from Q. agrifolia onto the less strongly defended leaves of Q. lobata if the leaves of this species contained lower tannin polyphenol concentrations.

The highest polyphenol levels reported in this study are higher than most reported in the literature (see Chapter 7.1) and higher than the mean levels in South African strandveld species (Chapter 5.2). These concentrations of polyphenols in oaks nevertheless declined to well within the range for strandveld, maquis and garrigue species as the leaves aged (Chapter 6.1). Such high levels may be related to heavy, cyclical defoliation on these oaks due to oakmoth outbreaks.

Although no inductive effect of increased polyphenol levels in successive cohorts of leaves was detected in this study, it has not been definitively ruled out. In order to support or refute induction in these oaks, it would be necessary to continue chemical testing of leaves and controlled experimentation for a number of seasons to include data from refractory periods in outbreak cycles. Induction has been suggested with slim evidence for Q. rubra in the northeast U.S.A.(Schultz & Baldwin 1982). More substantial evidence was found in a number of other studies (Green & Ryan 1972, Haukioja & Niemela 1978, Haukioja 1980, Bryant 198) as reviewed by Rhoades (1979) and, with additional evidence, Rhoades (1983). The spacing of outbreak cycles in P. californica in California might possibly be due to a coupling of climatic conditions and induced phytochemical changes : a mild drier winter would result in reduced larval mortality which, in turn, would allow rapid population increase in early spring(Harville 1955). The requisite physiological changes would then be initiated in the trees and this would eventually begin to accelerate mortality in the larvae. This type of model is a combination of ideas proposed by White (1974,1978,1984) and Haukioja and Hakala (1975). Although apparently able to cope with very high levels of polyphenols in Californian oaks, P. californica growth rates were significantly depressed when the larvae were fed artificial diet containing commercial tannin in concentrations comparable

to naturally occurring polyphenols (Puttick & Glyphis, unpublished data). In addition, the oaks are likely to produce a variety of tannin polyphenols not distinguishable by the methods used in this study, and use this chemical variation as an additional defence (Zucker 1983). Specific tannin polyphenols, far from being a general feeding deterrent, may actually be very specific and act in a dose-dependent manner (Zucker 1983, Hagerman & Butler 1981).

It has been proposed that herbivores may be able to partially circumvent the effects of high polyphenol levels on dietary protein availability. One solution in insects may be to elevate gut pH high enough to dissociate the protein-polyphenol complex (Goldstein & Swain 1965, Khatoon & Khan 1978, Berenbaum 1978). A second possible method of circumvention may be elevation of feeding rate and through-put time, to ensure adequate intake and digestion of sufficient protein (Slansky & Feeny 1977, Mattson 1980, Puttick submitted).

Other studies have found that increased leaf specific weight was an important factor in reducing consumption by invertebrate herbivores (Rausher 1981). Since sclerophylly represents a noticeable physical change in leaf appearance, some physiological changes also probably occur during sclerophyllyzation in any given leaf. These would, in turn be linked to changes in important chemicals in the leaf. For example, sclerophylly might include thickening of cell walls, increased fibrousness and lignification (Swain 1979): since condensed tannins are intermediate in the shikimate pathway that leads to lignin synthesis, one might predict that these compounds would co-vary with LSW. However, this was not the case: the best predictor of LSW in evergreen Q. agrifolia was decreasing nitrogen i.e. the higher the LSW the lower the concentration of N, while condensed tannins (proanthocyanidins) showed a very low correlation. In deciduous Q.

lobata total polyphenols appeared to be the best predictor: higher polyphenols correlated best with low LSW, yet condensed tannins (proanthocyanidins) did show a stronger positive correlation in this deciduous species than in the evergreen species. The difference in these correlations gives some indication of how the two oaks differ in their allocation of resources.

As described in the previous paper (Chapter 7.1), values for nitrogen (% d.w.) were converted to protein using a commonly used factor (6.25). This protein value was then incorporated in a 1 : 1 ratio with each of the three measures of polyphenols in order to approximate the formation of a polyphenol-protein complex (Feeny 1969). As expected and as described for evergreen and deciduous oaks in Chapter 7.1, the patterns in Q. agrifolia were quite different from those in Q. lobata. Seasonally, total polyphenols in Q. agrifolia were lowest compared with protein during midwinter (December through to March) and this broad pattern occurred with protein : astringency as well as protein : condensed tannins. During midwinter P. californica larvae would have depressed metabolism and low food intake and therefore defoliation pressure would also have been very low. In Q. lobata, however, polyphenol : protein ratios were lowest during midsummer (June and July). These trees did not have overwintering larvae and only became heavily infested once the overwintering larvae had pupated and the emerging moths had laid eggs on Q. lobata trees later in the summer. Oviposition occurred in late June and defoliation above 20 % on Q. lobata became apparent only in late July. In late July, the protein : polyphenol ratio began to decline and did so steadily to a minimum level in November. In other words, protein levels were decreasing and/or polyphenol levels were increasing. Protein : astringency followed a similar pattern but protein : condensed tannin declined steadily right from the beginning when new leaves first emerged in April. This change was occurring while larvae were in their final two

instars and would possibly result in higher larval through-put rates (see above).

The significant correlation between polyphenol levels and astringency in new Q. agrifolia leaves and the N content of those leaves, and the absence of any similar relationship in new leaves of Q. lobata , suggests an interesting distinction between these two species. The polyphenol levels in new leaves of both species are within the same range (12.0 - 18.0 % TAE d.w.), as are N levels (3.0 - 4.0 % d.w.). However, in mature leaves of Q. agrifolia, the lowest N levels are approximately 1 % while those of Q. lobata are double (approximately 2 %). Because of the course of Phryganidia's life cycle, new leaves of deciduous Q. lobata are not subject to the defoliation pressure of all Q. agrifolia leaves, which have been available to be used by some of the larvae for overwintering. The significant correlation of nitrogen and total polyphenols as well as astringency particularly in new Q. agrifolia leaves, suggests that this species is protecting its nutrient reserves which are generally lower in this evergreen species,in the leaves and hence its"photosynthetic apparatus" (Mooney & Gulmon 1982). No significant correlation was found in Q. lobata for nitrogen and polyphenols.

Insect herbivores consuming tree foliage have been proposed as regulators of primary productivity in forest ecosystems (Mattson & Addy 1975) and as important influences on nutrient cycling rates (Carlisle et al. 1966b, Springett 1978, Gorham et al. 1979). In a study concurrent with the present research, photosynthesis and water relations were examined in the same two species of oak prior to and during the outbreak of P. californica (Hollinger 1983). The average consumption was crudely estimated as 34.2 % of Q. agrifolia and 21.7 % of Q. lobata foliage production while consumption in peak seasons reached close to 100 %. Hollinger (ibid.) found these consumption figures to be high in comparison to those in some other systems. In temperate forest systems,

herbivores consume on average from 3 % to 11 % of foliage production (e.g. Bray 1964, Whittaker & Woodwell 1968, Petrusewicz & Grodzinski 1975, Whittaker et al. 1979, Ohmart et al. 1983). During outbreaks of Lepidoptera, this consumption of annual foliage production can increase to 24 %, such as when Quercus petraea was consumed by Tortrix viridiana (Carlisle 1966), or even 33 % when mixed hardwood foliage was consumed by fall cankerworm Alsophila pometaria (Waide et al. (unpublished data) in Swank et al. 1981). Hollinger (ibid.) found that the high consumption on Californian oaks led to high nutrient flow into the litter beneath them and suggested that the strong seasonal nature of precipitation would then result in substantial losses of nutrients from the system : there was some incidental evidence for reduced productivity and reproduction of oaks subsequent to heavy defoliation.

In summary, the chemical profiles of the leaves of an evergreen and a deciduous oak followed different patterns during the course of heavy defoliation by a lepidopteran herbivore. These different patterns suggested that these oaks represented a very different food resource for this herbivore.

Table 7.2.1 Means and standard deviations for three measures of tannin polyphenols on Quercus agrifolia and Q. lobata in successive years.

	1981		1982	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Total polyphenols (TAE % d.w.)				
Q. agrifolia (n = 12)	16.8	7.5	19.1	6.8
Q. lobata (n = 9)	17.0	2.4	19.7	5.7
Astringency (TAE % d.w.)				
Q. agrifolia (n = 12)	14.5	5.7	13.3	4.3
Q. lobata (n = 9)	18.8	3.8	14.7	2.2
Proanthocyanidins (QTE % d.w.)				
Q. agrifolia (n = 11)	4.8	3.5	3.9	2.4
Q. lobata (n = 6)	4.1	2.9	10.1	11.9

Table 7.2.2a. Correlation matrix between various measures of leaf chemical quality and leaf specific weight (LSW) in Q. agrifolia.

	N	P	H_2O	Tot.poly.	Astring.	Proantho.
Phosphorus	0.73					
H_2O content	0.54	0.65				
Tot. polyphenols	0.41	0.33	0.68			
Astringency	0.41	0.30	0.41	0.26		
Proanthocyanidins	-0.04	-0.13	-0.21	-0.15	-0.07	
LSW	-0.72	-0.67	-0.67	-0.56	-0.36	0.12

Table 7.2.2b. Stepwise multiple regression of various measures of leaf chemical quality on LSW of Q. agrifolia leaves. $df_1 = 6$, $df_2 = 55$, $F_{0.05} = 2.34$, $F_{0.01} = 3.29$.

Dependent variable	F	Partial F	Partial r	R^2	SD of resid.	SE as % of mean	B coeff.
Step 1 N	63.87	28.97	-0.72	0.52	0.0020	12.43	-0.0016
Step 2 H_2O	49.66	17.54	-0.67	0.63	0.0018	10.99	-0.0003
Constant							0.0409

Table 7.2.3a. Correlation matrix between various measures of leaf chemical quality and leaf specific weight (LSW) in Q. lobata.

	N	P	H_20	Tot.poly.	Astring.	Proantho.
Phosphorus	0.23					
H_20 content	0.18	0.45				
Tot. polyphenols	0.29	0.65	0.72			
Astringency	-0.17	-0.10	0.38	0.22		
Proanthocyanidins	-0.17	-0.12	-0.54	-0.32	-0.26	
LSW	-0.61	-0.46	-0.70	-0.73	-0.06	0.46

Table 7.2.3b. Stepwise multiple regression of various measures of leaf chemical quality on LSW of Q. lobata leaves. $df_1 = 6$, $df_2 = 29$, $F_{0.05} = 2.43$, $F_{0.01} = 3.50$.

Dependent variable	F	Partial F	Partial r	R^2	SD of resid.	SE as % of mean	B coeff.
Step 1 Tot. polyphenols	38.15	6.45	-0.73	0.53	0.0013	10.22	-0.0001
Step 2 Nitro.	39.07	26.57	-0.61	0.70	0.0010	8.23	-0.0012
Step 3 H_20	37.70	11.08	-0.70	0.78	0.0009	7.21	-0.0001
Constant							0.0337

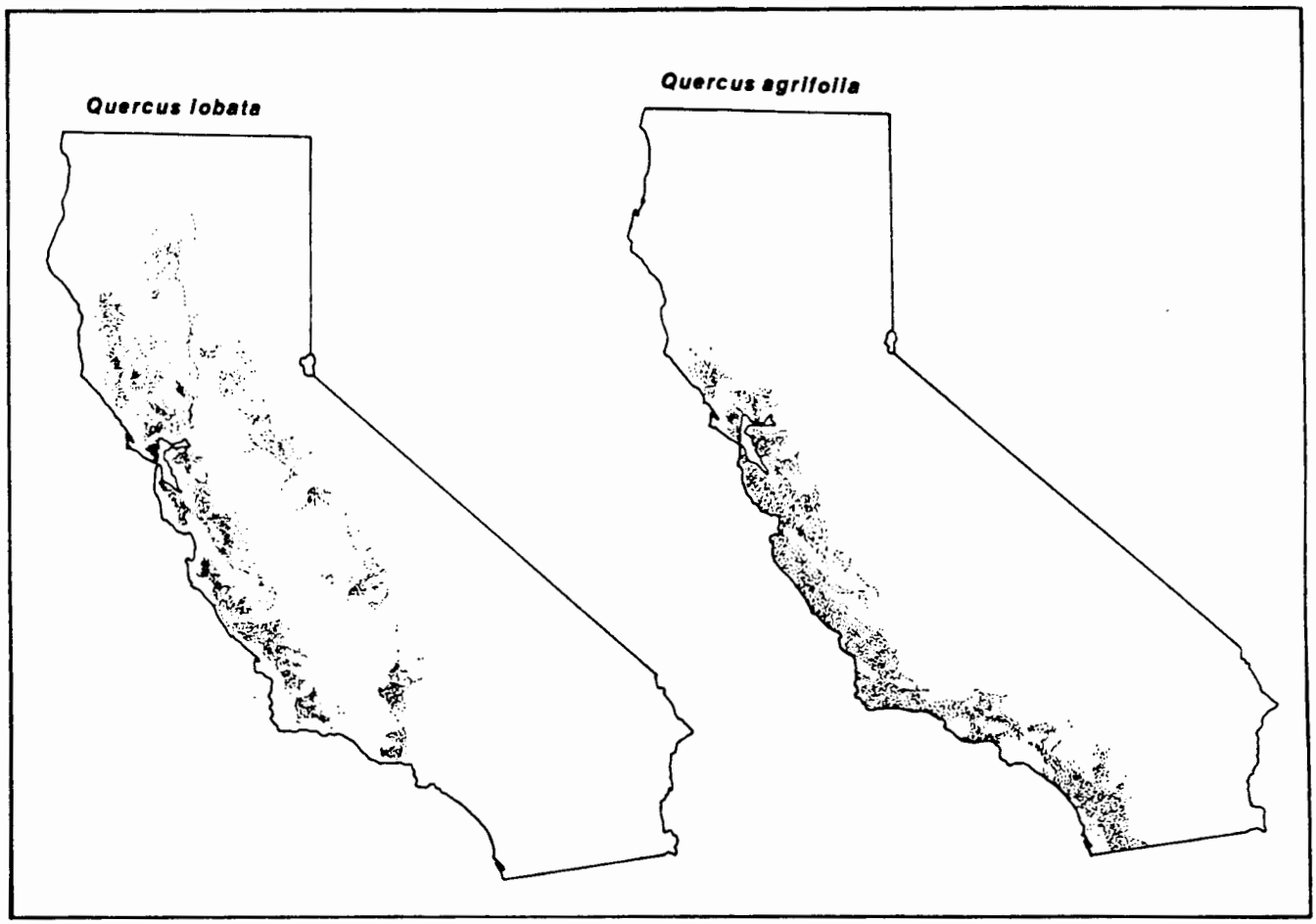


Figure 7.2.1. The distribution of Quercus lobata and Q. agrifolia in California(from Griffen & Critchfield 1972).

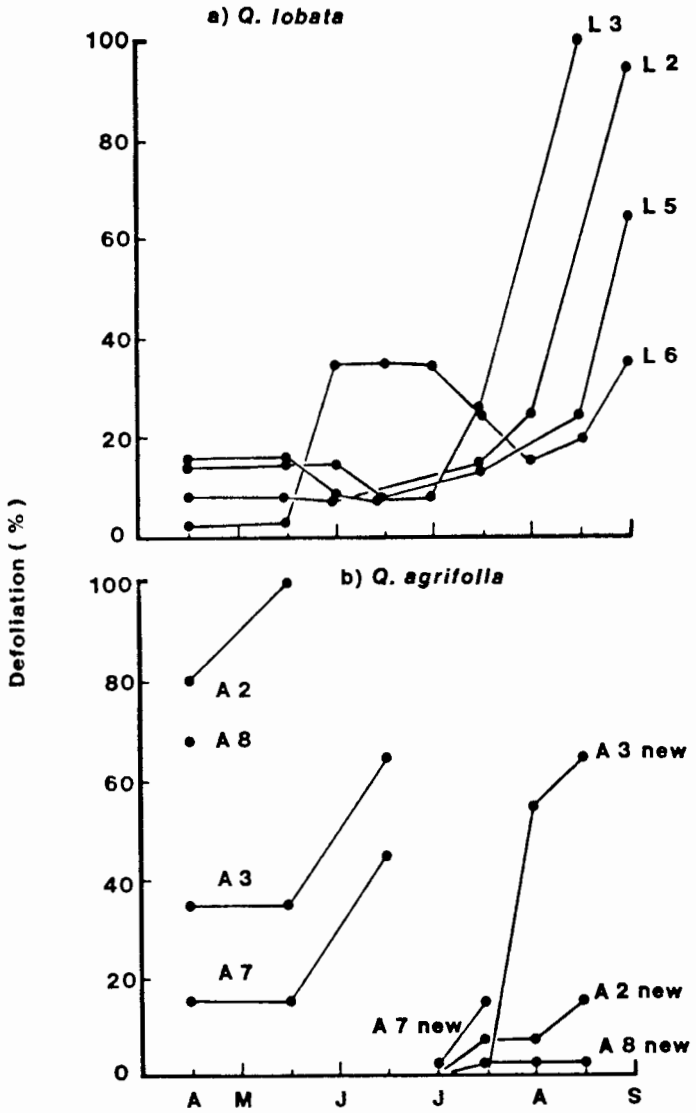


Figure 7.2.2. The progression of defoliation (% estimated by eye) on a) Q. lobata and b) Q. agrifolia from the end of April to the beginning of September 1981, during the peak of defoliation by Phryganidia californica.

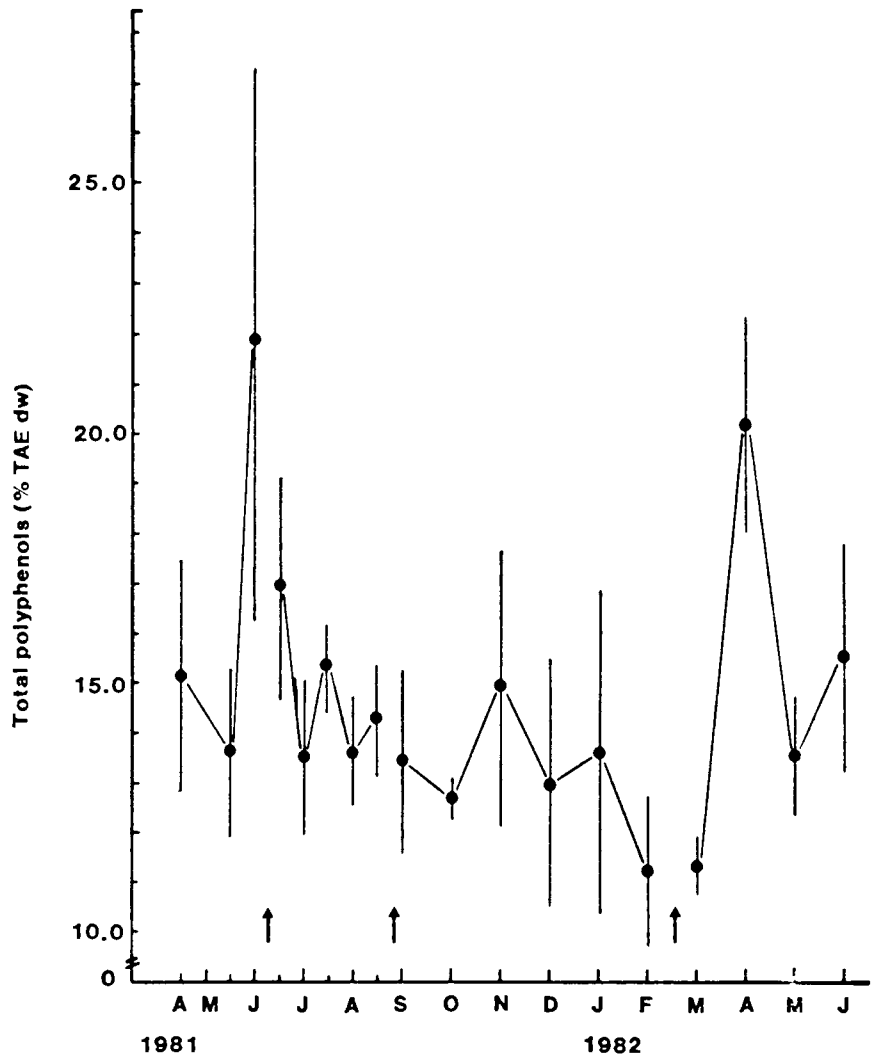


Figure 7.2.3 Seasonal variation in total polyphenols (% TAE d.w.) in Q. agrifolia (n = 4) from April 1981 - June 1982. Vertical bars indicate ± one standard error. Arrows indicate the initiation of new cohorts of leaves.

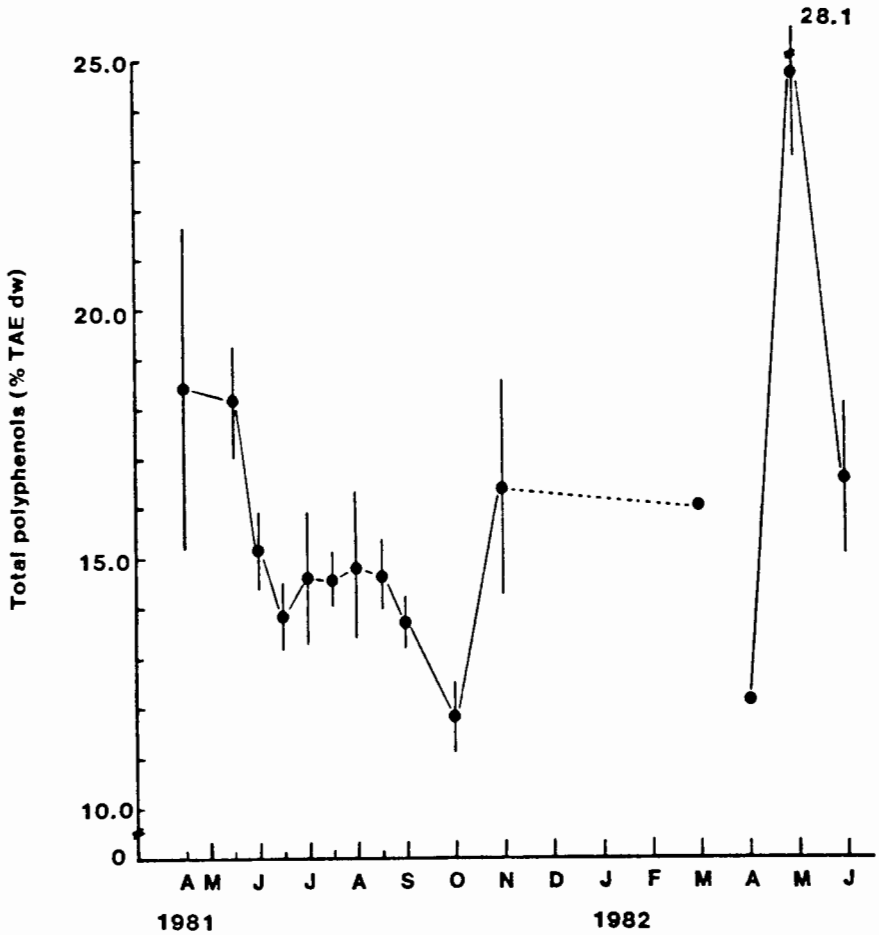


Figure 7.2.4 Seasonal variation in total polyphenols (% TAE d.w.) in Q. lobata (n = 4) from April 1981 - June 1982. Vertical bars indicate ± one standard error. The single observation and dotted line to March 1982 represent leaves which were not abscissed the previous fall.

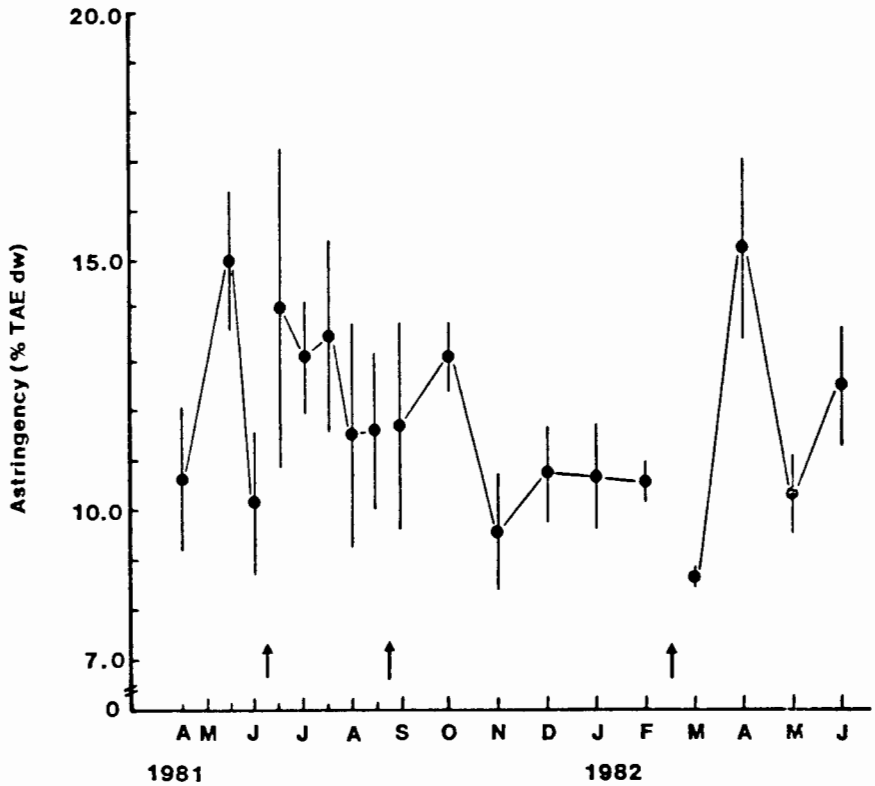


Figure 7.2.5 Seasonal variation in astringency (% TAE d.w.) in Q. agrifolia (n = 4) from April 1981 - June 1982. Vertical bars indicate ± one standard error. Arrows indicate the initiation of new cohorts of leaves.

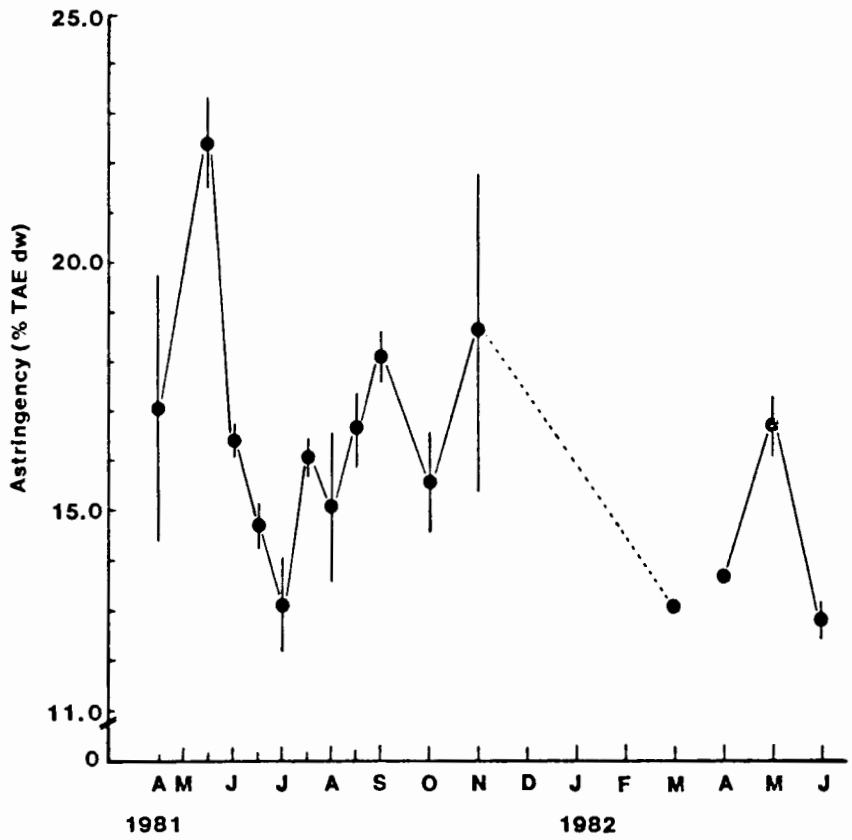


Figure 7.2.6 Seasonal variation in astringency (% TAE d.w.) in Q. lobata (n = 4) from April 1981 - June 1982. Vertical bars indicate ± one standard error. The single observation and dotted line to March 1982 represent leaves which were not abscissed the previous fall.

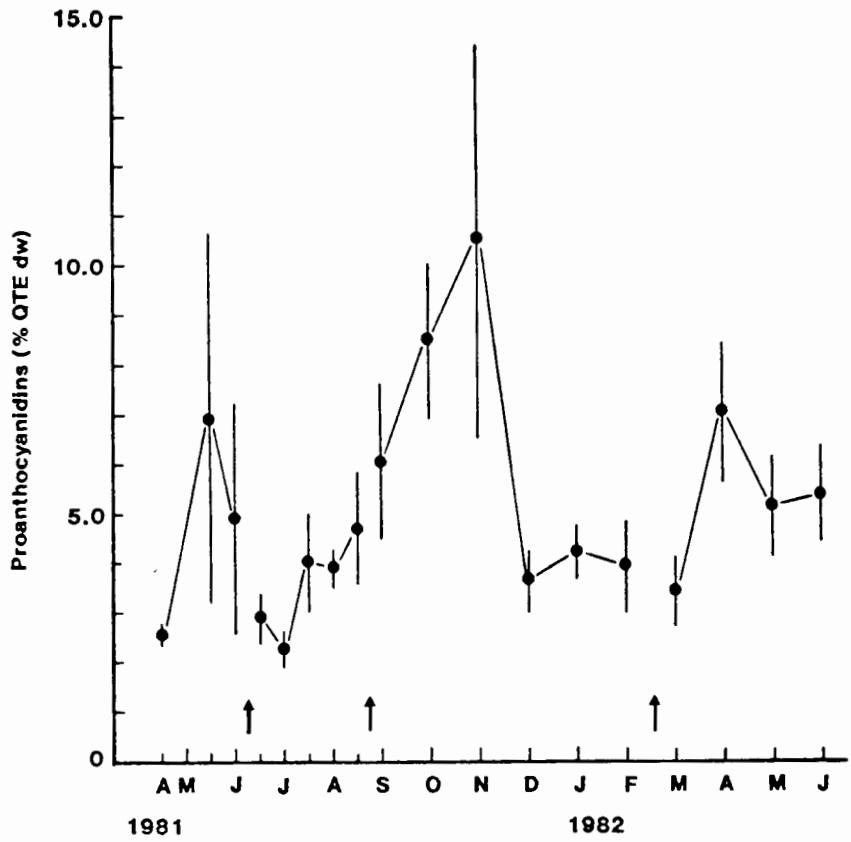


Figure 7.2.7 Seasonal variation in proanthocyanidins (% QTE d.w.) in Q. agrifolia (n = 4) from April 1981 - June 1982. Vertical bars indicate ± one standard error. Arrows indicate the initiation of new cohorts of leaves.

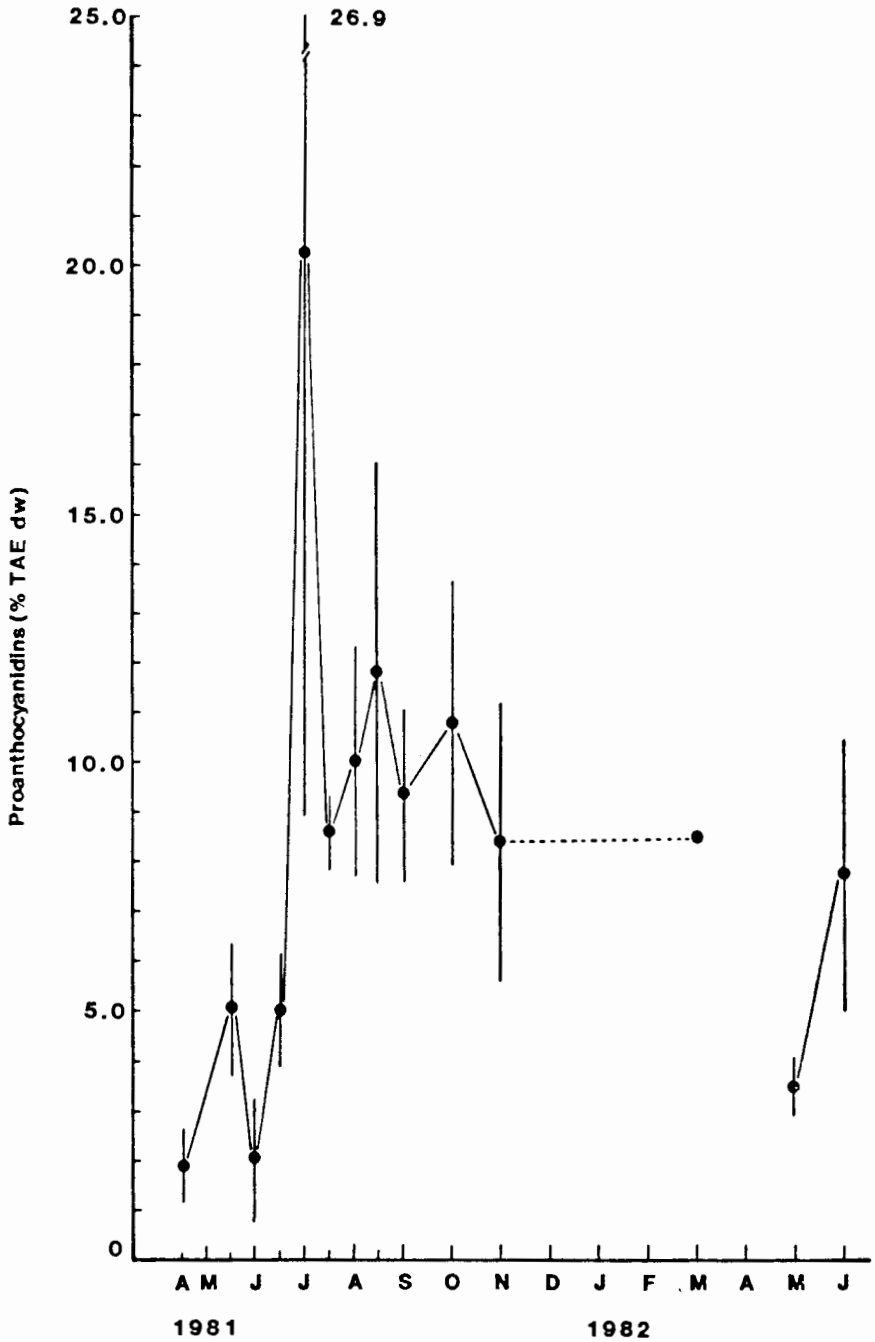


Figure 7.2.8 Seasonal variation in proanthocyanidins (% QTE d.w.) in Q. lobata (n = 4) from April 1981 - June 1982. Vertical bars indicate ± one standard error. The single observation and dotted line to March 1982 represent leaves which were not abscissed the previous fall.

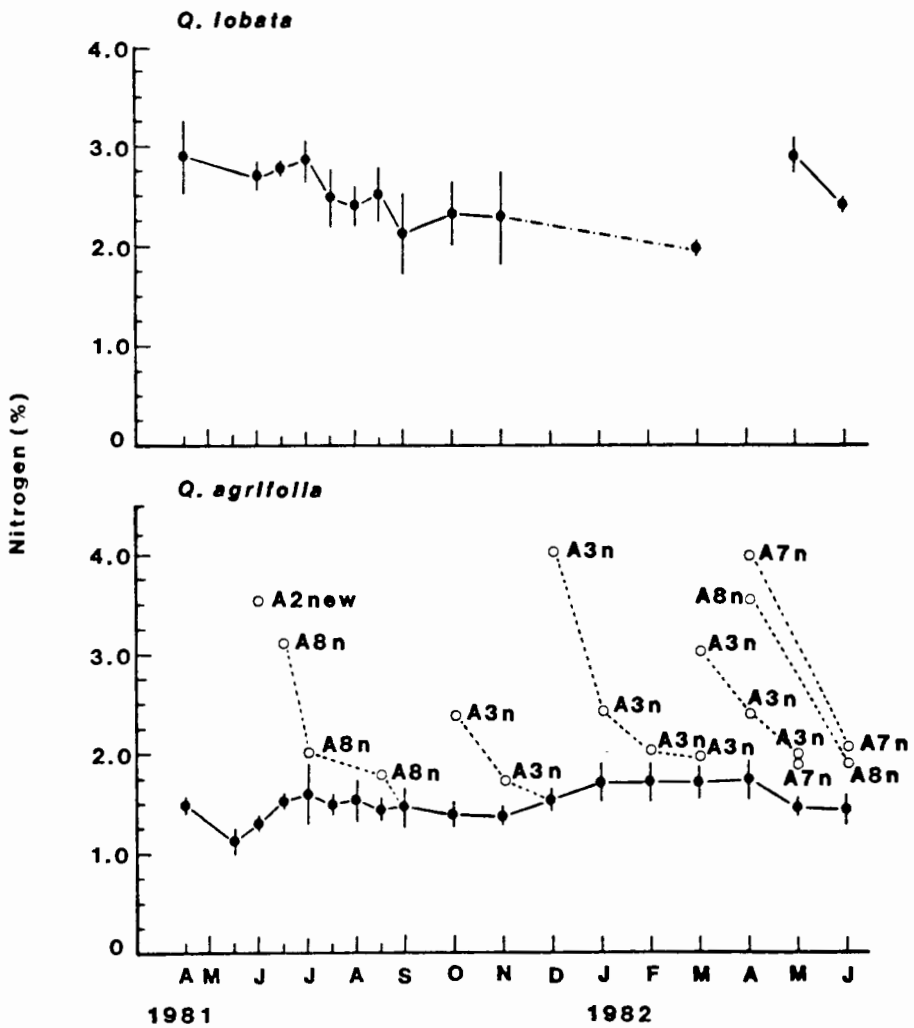


Figure 7.2.9 Seasonal variation in nitrogen (%) in Q. lobata (n = 4) and Q. agrifolia (n = 4) from April 1981 - June 1982. Vertical bars indicate ± one standard deviation. In Q. lobata the single observation and dotted line to March 1982 represent leaves which were not abscissed the previous fall.

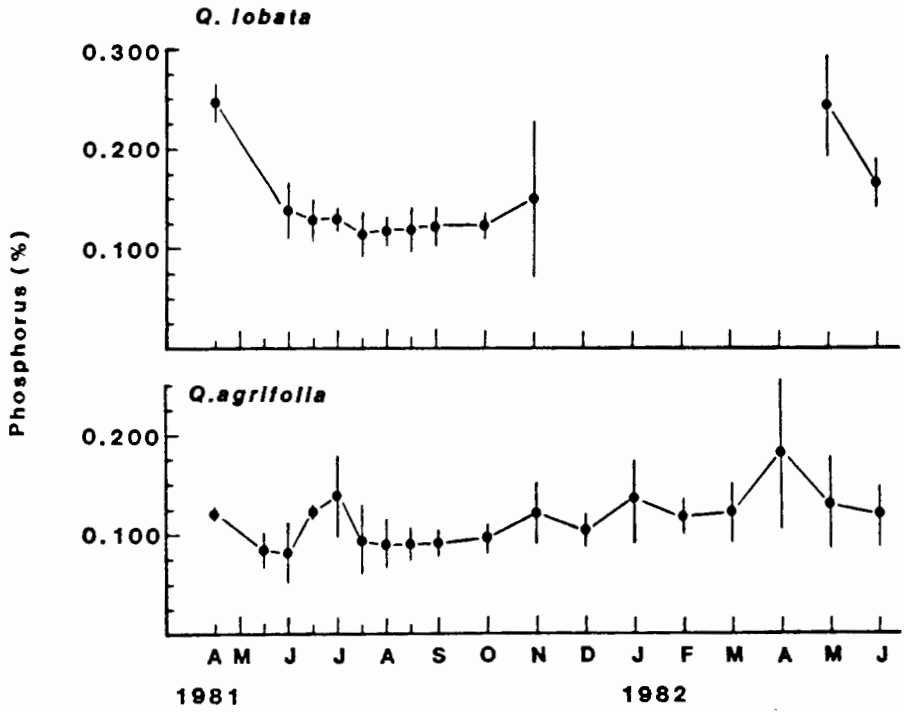


Figure 7.2.10 Seasonal variation in phosphorus (%) in Q. lobata (n = 4) and Q. agrifolia (n = 4) from April 1981 - June 1982. Vertical bars indicate ± one standard deviation.

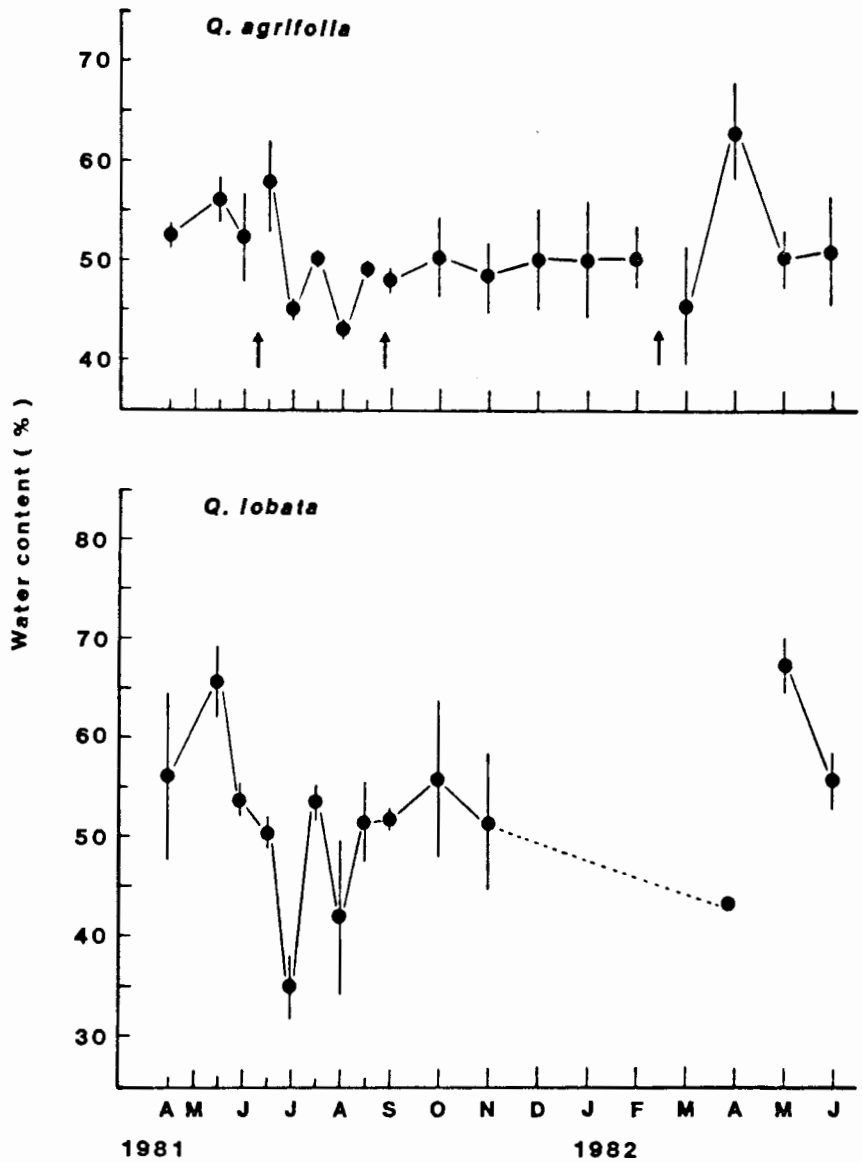


Figure 7.2.11 Seasonal variation in water content (% w.w.) in Q. agrifolia (n = 4) and Q. lobata (n = 4) from April 1981 - June 1982. Vertical bars indicate ± one standard deviation. In Q. agrifolia arrows indicate the initiation of new cohorts of leaves. In Q. lobata the single observation and dotted line to March 1982 represent leaves which were not abscissed the previous fall.

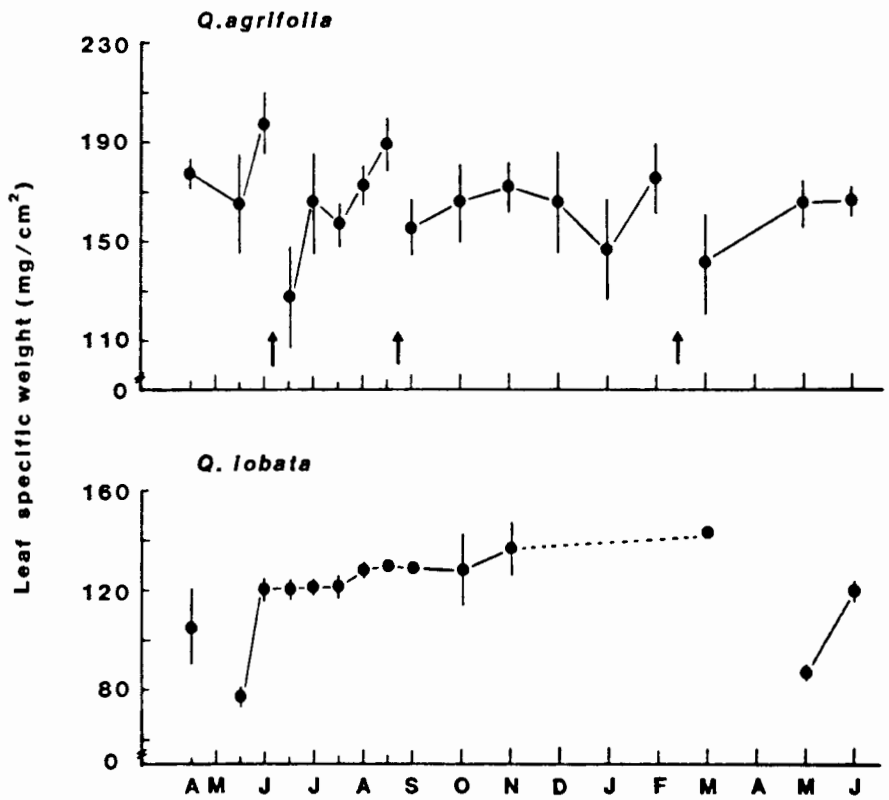


Figure 7.2.12 Seasonal variation in leaf specific weight (mg per cm^2) in Q. agrifolia (n = 4) and Q. lobata (n = 4) from April 1981 - June 1982. Vertical bars indicate ± one standard deviation. In Q. agrifolia arrows indicate the initiation of new cohorts of leaves.

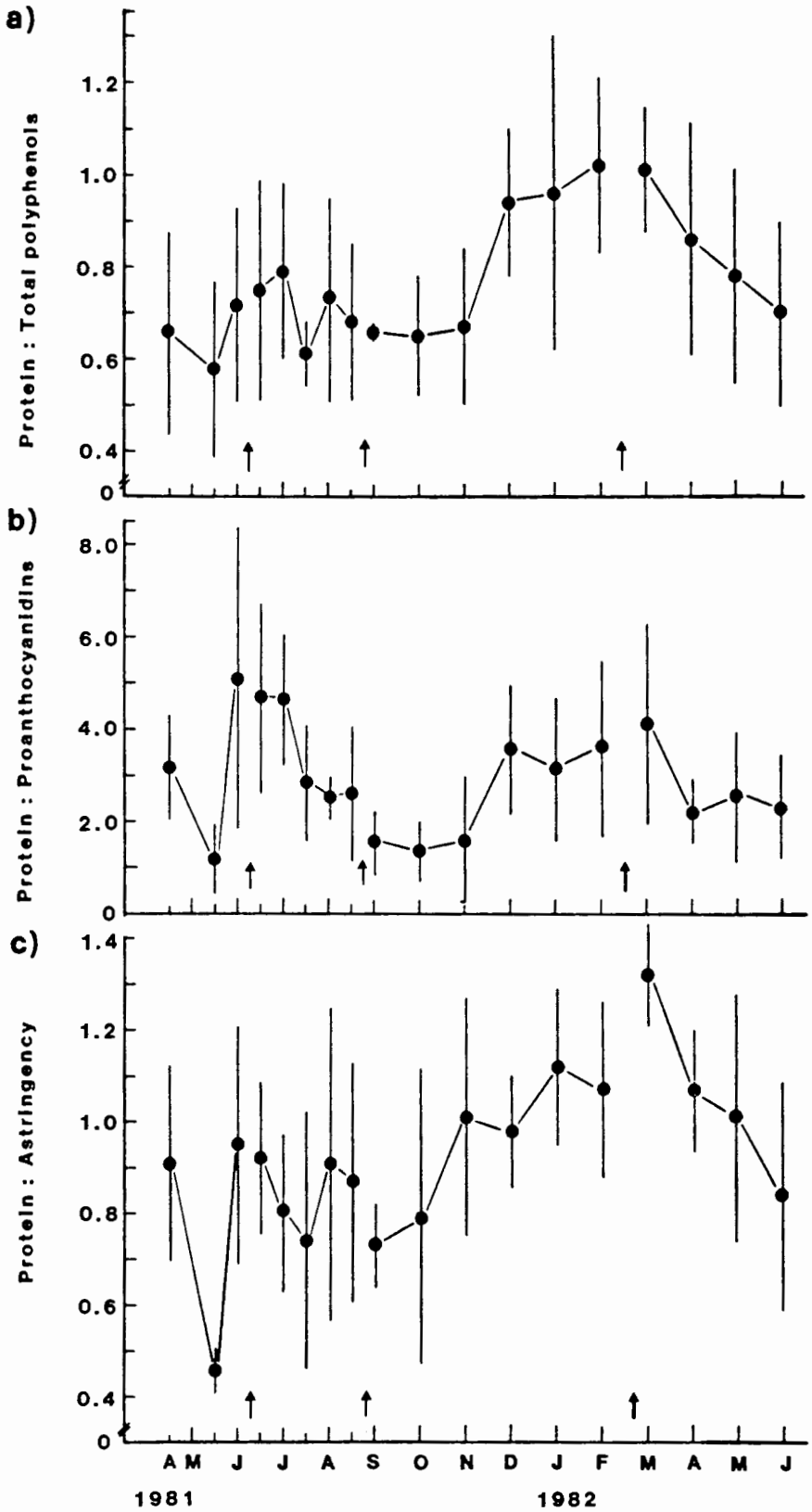


Figure 7.2.13 Ratio of protein (%) to a) total polyphenols (% TAE d.w.) b) proanthocyanidins (% QTE d.w.) and c) astringency (% TAE d.w.) in Q. agrifolia (n = 4) from April 1981 - June 1982. Protein was calculated from (N (%) x 6.25). Vertical bars indicate ± one standard deviation. Arrows indicate the initiation of new cohorts of leaves.

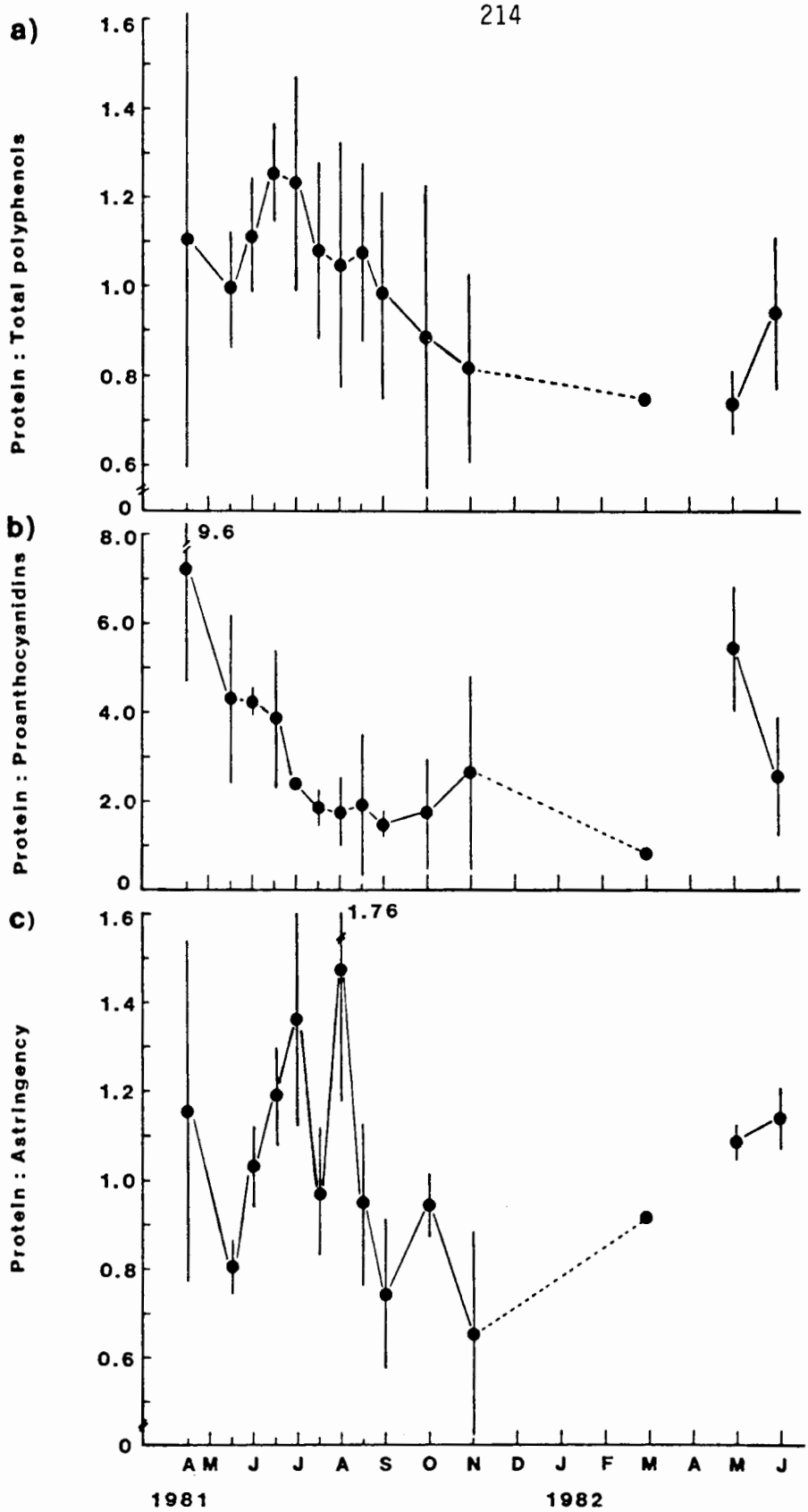


Figure 7.2.14 Ratio of protein (%) to a) total polyphenols (% TAE d.w.) b) proanthocyanidins (% QTE d.w.) and c) astringency (% TAE d.w.) in Q. lobata (n = 4) from April 1981 - June 1982. Protein was calculated from (N (%) x 6.25). Vertical bars indicate ± one standard deviation. The single observation and dotted line to March 1982 represent leaves which were not abscissed the previous fall.

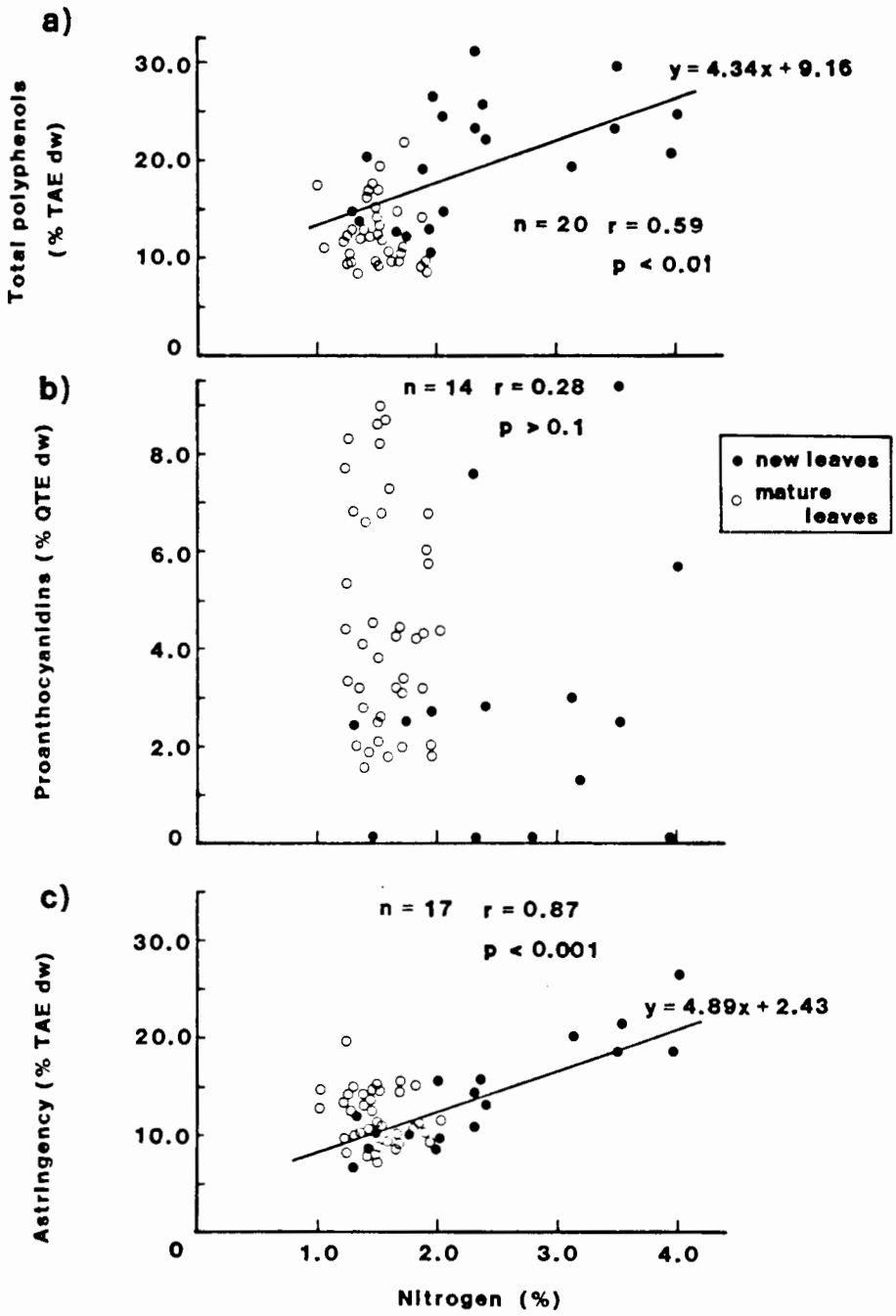


Figure 7.2.15 The relationship between a) total polyphenols (% TAE d.w.) b) proanthocyanidins (% QTE d.w.) and c) astringency (% TAE d.w.) and nitrogen (% d.w.) in Q. agrifolia. The regression equation comprises values for new leaves only, but values for mature leaves are included in the figure for comparison.

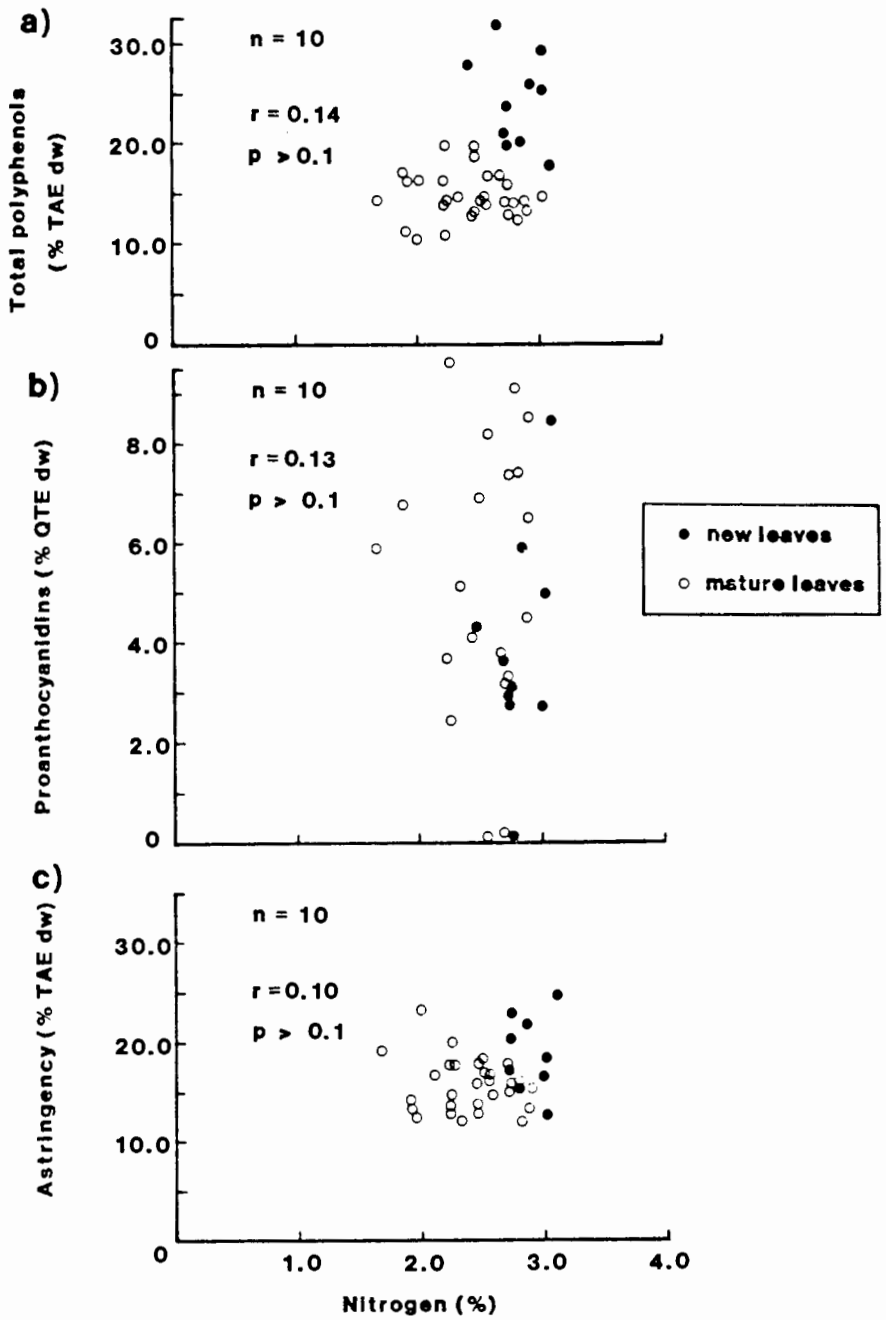


Figure 7.2.16 The relationship between a) total polyphenols (% TAE d.w.) b) proanthocyanidins (% QTE d.w.) and c) astringency (% TAE d.w.) and nitrogen (% d.w.) in Q. lobata. The regression equation comprises values for new leaves only, but values for mature leaves are included in the figure for comparison.

8.0 Synthesis

In this final section of the thesis, I propose to combine a summary of each chapter with some of the general and specific implications of the findings and to conclude with some ideas for further research.

Carbon, a major constituent of primary structural plant tissue, is also found in an enormous array of plant secondary metabolites. Many of these have been found to be herbivore feeding deterrents. Separately, the process of carbon gain from the atmosphere is closely connected to nutrients absorbed from the soil, particularly nitrogen (N). The five Mediterranean-type ecosystems around the world have some of the lowest soil nutrition levels of any ecosystems, specifically as measured in levels of N and phosphorous (P). Some whole plant and tissue culture studies have provided evidence that low substrate N levels stimulated an increase in the biosynthesis of some secondary metabolites from the Shikimate pathway, specifically polyphenolics. A large body of research has examined the ecological and physiological consequences of the capacity of polyphenolics to form insoluble complexes with proteins and other compounds important in animal diets. Separately from the inter-relationship between carbon gain and nutrition levels in the substrate, low substrate nutrition also translates into similarly low nutrition levels in the leaves of plants. Therefore, the combination of lower leaf nutrition and increased polyphenolic levels presents particular dietary problems for herbivores feeding on plants in mediterranean ecosystems. This thesis presents results from research on the diet of vertebrate and invertebrate herbivores and the quality of their food plants in mediterranean-type shrublands in South Africa, France and California.

High concentrations of leaf tannin polyphenols appear to be a characteristic of these three

mediterranean shrublands. In South African shrubland, three species of commonly occurring endemic small antelope (Raphicerus campestris steenbok, R. melanotis grysbok and Sylvicapra grimmia duiker) fed selectively on or avoided approximately 25 shrubland species. Their preference (quantified by means of a Jacobs D index of selectivity) was not correlated with the relative abundance of these plants in the vegetation but depended on other factors. Antelope shifted their preferences among different life-form categories coincident with phenological changes in the plant species in these categories. Observations of captive R. campestris and R. melanotis supported these findings. A selection of twelve plant species was chemically assayed for concentrations of different types of tannin polyphenols. The plant species which were selected were those which were either particularly abundant in the vegetation, or were obviously preferred or avoided as dietary items. Polyphenol assays included those for total polyphenols (tannic acid equivalents - TAE), proanthocyanidins (quebracho tannin equivalents - QTE), flavanols (catechin equivalents - CE) and astringency (TAE). Mean seasonal values for all tests increased from lowest concentrations in winter to highest concentrations in autumn. Total polyphenols (TAE) for individual species ranged very widely from 2.0 to 32.0 % d.w.. Concentrations in new and old leaves were not significantly different. Browse data, polyphenol data and data from proximate analyses (proteins, soluble carbohydrates, fats, fibre and ash) were assembled in a matrix: stepwise regression analysis of this data matrix indicated that total polyphenols and astringency were the strongest predictors of browse preference. This appears to be the first time that ruminant foraging preferences have been evaluated in this way. Vegetation biomass of this shrubland (1440 g m^{-2}) and production (360 g m^{-2} per year) were estimated from an exclusion plot experiment. These values were discussed in comparison with those from both mediterranean and other low nutrient ecosystems. In summary, the antelope feeding preferences in the South African shrubland were

strongly and negatively correlated with leaf tannin polyphenols than positively with any nutritional components (eg. protein, fats), using stepwise regression analysis. These antelope appear to have adjusted to high tannin levels by behavioural shifts in feeding preference.

In southern France, garrigue plant species growing on a higher nutrient calcareous substrate had higher foliar N levels than the same species growing on a lower nutrient siliceous substrate (maquis). However the leaves of the maquis species had significantly higher levels of P, more water, higher tannin polyphenol concentrations and larger leaf areas. The amount of insect damage on garrigue and maquis plants was similar, presumably due to different nutritional "advantages" in each case. Artificial soil fertilization significantly elevated N levels in Q. coccifera and increased total leaf areas, and these leaves had significantly more insect damage. A combination of controlled stock utilization and artificial fertilization significantly lowered the levels of condensed tannins in Q. coccifera and raised the levels of foliar N. Some effects of burning on Q.coccifera are also described. This evidence from garrigue and maquis in southern France suggests that in these shrublands, artificial soil fertilization can render leaf material more nutritional by increasing nitrogen content and decreasing condensed tannin concentration, although very heavy grazing pressure increased the levels of leaf polyphenols.

Finally, in two evergreen (Q. agrifolia and Q. durata) and three deciduous Californian oak species (Q. lobata , Q. douglasii and Q. kelloggii) total polyphenol concentrations varied from 8.5 % d.w. (TAE) in Q. kelloggii in late summer to 27.5 % d.w. (TAE) in Q. durata in late summer during an outbreak of a lepidopteran herbivore, Phryganidia californica . Condensed tannins increased in all species from spring to late summer. In a

comparison of larval mass of P. californica collected from all five oaks simultaneously, larvae growing on evergreen oaks were significantly smaller than those growing on deciduous oaks. Stepwise regression analysis indicated that phosphorous and astringency predicted caterpillar mass most strongly. Tannin polyphenols and nutritional components of the leaves of one evergreen and one deciduous species were measured at regular intervals for 15 months during the P. californica outbreak. Two complete defoliations of Q agrifolia induced these trees to produce three cohorts of new leaves during that season. The deciduous Q. lobata were defoliated once by the end of the growing season and some trees produced new leaves before leaf abscission. In Q. agrifolia highest mean levels of tannin polyphenols were 21.0 % d.w. (TAE) and declined to a minimum of 11.0 % d.w., and in Q. lobata maximum mean levels in new leaves were 18.5% d.w. (TAE) and declined as the leaves aged to 12.0% d.w. before abscission: this is contrary to findings from other oak - lepidopteran studies. As mentioned in Chapter 7.2, levels as high as these exceed the apparent threshold of tannin concentration above which oak moth larval growth was depressed. Condensed tannins generally increased in both species as leaves aged. There was no evidence for short or long term inductive effects in subsequent cohorts of leaves. Inductive effects have been described in other studies. Leaf nitrogen levels declined from a maximum in new leaves of 3.0 to 4.0 % d.w. in both species to approximately 1.0 % d.w. in Q. agrifolia and 2.0 % d.w. in Q. lobata . This implies that P. californica may some adaptive mechanism, possibly physiological, for coping with these compounds and their chemical impact on the nutritional quality of leaves.

These findings contribute significantly to knowledge of the ecological biochemistry of plant-animal interactions in three major areas: food quality and ruminant food preferences, changes in food quality during insect population outbreaks and the

sensitivity of food quality to substrate nutrition. Food quality has been central to a number of debates about animals and resource utilization for at least the past two decades. A more recent debate about whether secondary metabolites or food quality is the regulating factor in invertebrate (Frankel 1959) and vertebrate food preferences (Bryant 1981) is predated by a similar controversial discussion about whether herbivores are food limited or not (Hairston et al. 1960). In the criticism of this older argument, variation and heterogeneity in different aspects of host plants gradually emerged as a factor which had been overlooked (Denno & McClure 1983). In fact, the older debate appears to have been transformed and refined into the newer one about the importance of secondary metabolites versus nutritional components. The results of the antelope research suggest that secondary metabolites are more important in determining feeding preferences. However, with recent improvements in the analysis of nutritional compounds in general and a more concise definition of fibre in particular, it may be that subtle and complex combinations of metabolites and nutrients in certain conditions determine food choice. Tannin polyphenols are particularly important and interesting in this regard because of their interaction with proteins. This interaction occurs at two levels during feeding. First, these compounds have a specific taste and then subsequently, they complex both with ingested protein as well as with digestion enzymes. This means that avoidance of plants with higher tannin concentrations may be controlled by taste factors prior to any nutritional factors coming into play. In addition, very little research has been published on the formation of tannin-carbohydrate complexes and nutriton, and these may be just as important.

The results of a few herbivory studies, particularly where digestibility-reducing compounds such as tannin polyphenols were thought to be the important deterrent compounds, suggested that these compounds were ineffective as herbivore defences (eg.

Fox & Macauley 1977, Bernays 1978, 1981, Bernays & Woodhead 1982, Morrow & Fox 1980). These studies all dealt with insects which were specialists on their hostplants. Among vertebrates, one study has shown that woodrats Neotoma fuscipes had adapted to oak foliage which contained high concentrations of tannin polyphenols (Atsatt & Ingram 1983). These authors, in contrast with some of those cited above, did not conclude that the theory should be re-examined. Rhoades (1983) pointed out that no improved theoretical synthesis arises from these apparently conflicting studies which better explains the observed patterns. It will be necessary for critics to assemble a coherent set of findings into a more plausible theory with testable hypotheses before any currently useful theories are abandoned. In addition, Zucker (1983) notes that biochemical and chemical considerations have been virtually absent in this continuing debate. The findings described in this thesis support a large body of research which have found tannin polyphenols to be deterrent to herbivores.

The distribution of defenses among plant species has both ecological and evolutionary significance for feeding patterns and population dynamics of herbivores on the one hand and the success of the plants on the other. Theories of plant - animal interactions have originated mostly from research conducted in temperate systems on insects (Feeny 1968, 1970, McKey 1975, Feeny 1976, Rhoades & Cates 1976, McNeill & Southwood 1978). This theory has tended to emphasize the animal end of the interaction e.g. animal responses at the population level to chemical compounds. Most of the insect research has continued in temperate systems while much of the work on vertebrates has been conducted in stressed, low-nutrient environents (tropics and the sub-arctic). This largely insect-derived theory may well be inappropriate or at least insufficient for predicting the responses of vertebrate herbivores for a variety of reasons.

First, in terms of scale vertebrates may browse the end of twig which might include both a cohort of new leaves as well as the primordia for the stem and other new leaves. Some vertebrates may have very specific search images for particular parts of a plant e.g. the colobine monkeys which select leaf petioles, but the act of consuming such an item will have destroyed the leaf. Insects, however, may be as specific as leaf miners which eat only one layer of palisade mesophyll in a leaf. The process of gall formation seems to be non-interfering in the general physiology of the leaf, once the gall has developed. The degree of damage by insects may only equal that of vertebrates when outbreaks with widespread defoliation occur, and these are usually cyclical and unpredictable. Second, the most generalised insect is probably still more restricted in its plant species preferences and utilization patterns than the most specialised vertebrate. This is particularly important in relation to the theory of qualitative and quantitative defense chemicals (Rhoades & Cates 1976) and the relevance of generalised or specialised diet. Third, life cycle lengths and life history are obviously quite different in the two groups. Insect life spans are usually shorter than the life span of the plant material which constitutes their diet, or at least their life cycles are cued to being able to use the resource optimally in a small time frame. Insects are also capable of developing resistance to specific plant defence chemicals fairly rapidly due to short generation times. Vertebrates will continue to use the same plant species and individuals for years and at times when the species may be physiologically vulnerable. Finally, vertebrates are capable of learning by individual experience and by transmission (young learning by imitation of adults) and this is very important in food search. Insects appear to be able to detect very subtle differences in plant tissues but their responses are relatively inflexible. By being highly mobile compared to insects, vertebrates are capable of acting as much more persistent and general selective agents whereas insects are more confined. It is hoped that cognisance of these differences would lead to more rigorously defined hypotheses

and more robust theory.

Some interesting problems worthy of more attention have become apparent from this research. The South African mediterranean shrubland with its endemic small antelope offers a unique system in which to study mammalian feeding preferences. An extension of this study would be the establishment and cultivation of experimental plots which would contain a reduced set of the plant species found in the natural community. Each plot would contain representative plants with, for example, high and low tannin polyphenols levels, high and low fibre, high and low protein as well as a section of natural vegetation. Captive antelope would then be released into these plots and their preferences, consumption and performance could be monitored both on the selected plant species as well as on the adjacent natural vegetation. Soil fertilization of some plots or even individual plants, would present another very interesting facet in this programme, with obvious agricultural significance. With fistulation of these animals, it would be possible to sample stomach contents as well as administer nutrient and secondary compound solutions and then to monitor feeding behaviour and preferences. Fistulation sampling would make it possible to monitor changes in the gut microflora and to determine how the composition and size of these microbial populations are affected by different plant secondary metabolites. This area of secondary chemistry research has been largely confined to work on laboratory animals.

Some research questions appropriate to garrigue and maquis would be examination of seasonal changes in leaf chemical and physical properties in plants under different fertilization regimes. Particular insect herbivores could be identified and some field and laboratory experiments conducted to determine their responses to different combinations

of plant chemicals. It would be interesting to observe domestic and wild herbivores (hares perhaps) to watch selection behaviour and determine their preferences when offered a choice of species grown under different fertilization regimes. This last aspect should be supplemented with balance studies.

The most important extension of the oak moth research would be collection and analysis of oak leaves during a refractory period between outbreaks of the oak moth larvae or any other lepidopteran herbivore. From this chemical information it would be possible to establish whether or not induction occurs in these plants. In addition, a more detailed analysis of the specific tannin polyphenols occurring in these oaks would help both to elucidate their potential physiological effects on insect herbivores and to explain how the oak moth in particular avoids or resists these effects (Zucker 1983). Finally, having documented changes in the chemical quality of oaks during an outbreak, it would be interesting to determine the potential effect that such fluctuations might have on the population dynamics of the oak moth.

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ADDENDUM TO TABLE 5.4.1.

Since one of the examiners suggested that the design of the exclusion plot experiment was pseudoreplicated, I ran an ANOVA on the results from each of the four experimental plots. These results are given below.

	INSIDE		OUTSIDE			
	MEAN	± S.D.	MEAN	± S.D.	F	p
	$Kg.m^{-2}$ D.W.		$Kg.m^{-2}$ D.W.			
Plot 1						
Phytomass	0.44	0.28	0.43	0.26	0.01	>0.1
Wood	0.80	1.05	0.45	0.40	2.14	>0.1
Total	1.25	1.25	0.88	0.63	1.46	>0.1
Plot 2						
Phytomass	0.29	0.30	0.15	0.12	4.14	<0.050
Wood	0.61	1.13	o.23	0.16	2.39	>0.1
Total	0.90	1.40	0.39	0.26	2.88	>0.1
Plot 3						
Phytomass	0.40	0.43	0.33	0.30	0.39	>0.1
Wood	1.07	1.22	0.74	1.42	0.67	>0.1
Total	1.47	1.58	1.08	1.56	0.70	>0.1
Plot 4						
Phytomass	0.73	0.72	0.49	0.42	1.87	>0.1
Wood	1.40	1.18	0.69	0.70	5.92	<0.025
Total	2.13	1.77	1.18	1.04	4.74	<0.050